INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

DECIPHERING MICROGLIA PLASTICITY IN GLIOMA

Dalel Saidi

Karolinska Institutet

Stockholm 2019

The cover image shows the cross-talk between glioma (yellow) and microglia (blue) cells.

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by E-print AB

ISBN 978-91-7831-322-8

Deciphering Microglia Plasticity in Glioma

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Lecture Hall Föreläsningssalen Atrium, Nobels väg 12B, Karolinska Institutet Stockholm

Friday February 1st, 2019 at 9:30

By
Dalel Saidi

Principal Supervisor:
Bertrand Joseph
Karolinska Institutet
Institute of Environmental Medicine,
Unit of Toxicology

Co-supervisor(s):
Ola Hermanson
Karolinska Institutet
Department of Neuroscience

Nina Heldring
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Immunology

Opponent:
Michel Salzet
Lille 1 University of Science and Technology
Department of Biology

Examination Board:
Sven Nelander
Uppsala University
Department of Immunology, Genetics and Pathology

Karin Broberg
Karolinska Institutet
Institutet för miljömedicin

John Inge Johnsen
Karolinska Institutet
Department of Women's and Children's Health

To my parents and my husband

.

قال الله تعالى
وقُل رَّبِّ زِدْنِي عِلْمًا.
(طه : 114)

“Half of the secrets of the cell are outside the cell.”

Dr. Mina J. Bissell

Abstract

Microglia are the innate immune cells of the brain. One of the main characteristics of microglia is its extremely plasticity, which is necessary for them to deal with changing circumstances. Activation of microglia can contribute to contrasting effects by either pro-inflammatory, anti-inflammatory, or tumor-supportive phenotypes. Adult and pediatric high-grade glioma are very aggressive tumors with a median survival of less than a year. The pathological hallmark of these tumors is the invasion into brain tissue, which contributes significantly to the failure of current therapeutic treatments. Glioma cancer recruits microglia for their expansion and invasiveness. During the course of the disease, glioma-infiltrating microglia undergo cellular reprogramming and functional changes that are controlled by specific intracellular signaling pathways and epigenetic mechanisms.

In the first study, we describe a novel epigenetic pathway associated with microglia reprogramming toward the tumor-supporting phenotype that is of benefit to glioma biology. We demonstrate that the glioma-induced microglia tumor-supporting phenotype is coupled with a functional interaction between sirtuin 1 (SIRT1) and the specific H4K16 histone acetyltransferase hMOF, which leads to an increase of the global histone 4 lysine 16 (H4K16) acetylation. During the process of microglia reprogramming toward the tumor-supporting phenotype, we show that SIRT1 deacetylates hMOF that promotes its recruitment to the chromatin and enhances its enzymatic activity against H4K16 acetylation. The high enrichment in H4K16 acetylation results in an aberrant gene expression in tumor-supporting microglia. Furthermore, we show that the manipulation of H4K16's acetylation level, controlled by the enzymes hMOF and SIRT1, has an impact on microglial pro-tumoral activation.

Diffuse intrinsic pontine glioma (DIPG) is a pediatric high-grade glioma (pHGGs) that carries H3K27M mutation and has a reduced global level of the repressive posttranslational histone modification H3K27me3. In the second study, we explore the microglia profile in pHGGs and DIPG. Our research work suggests that microglia activation towards a tumor-supporting phenotype exhibits a significantly decreased level of H3K27me3 and demethylase JMJD3. Interestingly, the inhibition of EZH2 or JMJD3/UTX enzymes reduces the switch of microglia into pro-tumoral phenotype, which in turn has a negative effect on DIPG and pHGGS invasion.

In the third study, we thoroughly describe a completely novel molecular mechanism used by glioma cells to transform microglia into a tumor-supporting phenotype. We demonstrate *in vitro* and *in vivo* that decreased basal caspase-3 activity in microglia is a necessary condition for their polarization into a tumor-supportive phenotype. We reveal that nitric oxide originating from the glioma's nitric oxide synthase-2 induces the inhibition of microglial thioredoxin-2 denitrosylation activity, which in turn leads to an increased S-nitrosylation of caspase-3.

LIST OF SCIENTIFIC PAPERS

I. **Glioma-induced SIRT1-dependent activation of hMOF histone H4 lysine 16 acetyltransferase in microglia promotes a tumor-supporting phenotype**
Dalel Saidi, Mathilde Cheray, Ahmed M. Osman, Vassilis Stratoulias, Olle R. Lindberg, Xianli Shen, Klas Blomgren, and Bertrand Joseph
Oncoimmunology. 2018; V7(2): e1382790.

II. **Inhibition of EZH2 or JMJD3 reduces microglial pro-tumoral activation in Diffuse Intrinsic Pontine Glioma**
Dalel Saidi, Lara Friess, Bertrand Joseph
MANUSCRIPT

III. **Glioma-induced inhibition of caspase-3 in microglia promotes a tumor-supportive phenotype.**
Shen X, Burguillos MA, Osman AM, Frijhoff J, Carrillo-Jiménez A, Kanatani S, Augsten M, **Saidi D**, Rodhe J, Kavanagh E, Rongvaux A, Rraklli V, Nyman U, Holmberg J, Östman A, Flavell RA, Barragan A, Venero JL, Blomgren K, Joseph B.
Nature Immunology. 2016, 17(11):1282-1290.

CONTENTS

LIST OF ABBREVIATIONS

2-HG	R(-)-2-hydroxyglutarate
α-KG	α–Ketoglutarate
Aß	Amyloid Beta
AD	Alzheimer's disease
Arg1	Arginase 1
ATM	Ataxia – telangiectasia mutated
BBB	Blood brain barrier
BM	Bone marrow
BTSC	Brain tumor stem cell
Caspases	Cysteine dependent aspartate directed proteases
ChIP	Chromatin immunoprecipitation
cIAPs	Cellular inhibitor of apoptosis proteins
CNS	Central nervous system
coIP	Co-immunoprecipitation
CSF-1	Colony-stimulating factor 1
Cys	Cysteine
DIPG	Diffuse intrinsic pontine gliomas
ECM	Extracellular matrix
EGFR	Growth factor receptor
EGFRv III	Growth factor receptor variant III
EZH2	Enhancer of zeste homolog 2
FBS	Fetal bovine serum
GBM	Glioblastoma multiform
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSCs	Glioma stem cells
H3K27me3	Histone 3 lysine 27 trimethylation
H3K9ac	Histone 3 lysine 9 acetylation

H4K16ac	Histone 4 lysine 16 acetylation
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDMs	Histone demethylases
hMOF	Human orthologue of the *Drosophila melanogaster* gene males absent on the first
HMTs	Histone methyltransferases
IDH	Isocitrate dehydrogenase
IFN-γ	Interferon gamma
IGF-1	Insulin-like growth factor 1
IKK	IkB Kinase
IL4	Interleukine 4
IL6	Interleukine 6
IL13	Interleukine 13
IL-34	Interleukin 34
IL-10	Interleukin 10
IL-1ß	Interleukin 1 beta
JmjC	Jumonji C
JMJD3	Jumonji domain-containing 3
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MDSCs	Myeloid-derived suppressor cells
MEFs	Mouse embryonic fibroblasts
MFG-E8	Milk fat globule epidermal growth factor 8
MGMT	O6-methylghuanine DNA methyltransferase
MMPs	Matrix metalloproteases
MSL	Male specific lethal complex
NK	Natural killer
NES	Nuclear export signals
NLS	Nuclear localization signals

NO	Nitric oxide
NOS	Nitric oxide synthase
NPCs	Neuronal precursor cells
PcG	Polycomb group
PD	Parkinson's disease
pHGGs	Pediatric high-grade glioma
PKC-δ	Protein kinase c-d
PLA	*In situ* proximity ligation assay
POSTN	Periostin
PRC2	Polycomb repressive group
PS	Phosphatidylserine
RTK	Receptor tyrosine kinase
SIR2	Silence information regulators
siRNA	Small interfering RNA
SIRT1	Sirtuin 1
SIRT7	Sirtuin 7
TAM	Tumor associated macrophage / macroglia
TLR4	Toll-like receptor 4
Treg	Regulatory T cell
Trx	Thioredoxin
TSS	Transcriptional start site
UTX	Ubiquitously transcribed tetratricopeptide repeat X
WHO	World Health Organization

1. INTRODUCTION

1.1 GLIOMA

The central nervous system (CNS) is composed of the brain and the spinal cord. The brain is populated by different cell types that can be classified into neuronal and glial cells. Glial cells are further classified into astrocytes, oligodendrocytes and microglia, which are involved in maintaining the brain's homeostasis. The brain is an organ isolated from the peripheral immune system due to the presence of the blood brain barrier (BBB) and the absence of lymphatic vessels. Under certain conditions such as inflammation, the BBB becomes impaired and permeable to monocytes and other immune cells, and may allow larger molecules to enter the brain (Ballabh et al. 2004).

Brain tumors are characterized by high morbidity and mortality. They are classified into primary central nervous system tumors and secondary metastatic brain tumors that are derived mostly from lung and breast cancer. Primary brain tumors are a heterogeneous group of tumors arising from cells within the brain parenchyma and meninges.

Glioma is the most common malignant primary CNS tumor and remains the most difficult cancer to treat. Indeed, gliomas arise from the neuroglial stem or progenitor cells and are responsible for the majority of deaths from primary brain tumors. They account for 28% of all brain tumors and 80% of malignant tumors (Weller et al. 2015).

A glioma is diagnosed with an MRI upon development of symptoms such as seizures and focal neurological deficits. Diagnosis is based on histological classification of the tumors and by the World Health Organization (WHO) grading of malignancy from low to high, grade I to IV. In adults, gliomas include pilocytic astrocytomas, pleomorphic xanthoastrocytomas, and ependymomas that are less common and have a better prognosis. Moreover, Gliomas, that occur most frequently, include oligodendrogliomas and the controversial group of mixed oligoastrocytomas as well as infiltrative astrocytomas of various grades such as diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), and glioblastoma (WHO grade IV) (Louis et al. 2007). In young adults, pilocytic astrocytomas and diffuse midline gliomas such as diffuse intrinsic pontine gliomas of various grades are the most common types (**Table 1**).

The WHO classification, updated in 2016, is based on genetic alterations combined with histology. According to the 2016 WHO classification, gliomas are grouped into circumscribed gliomas and diffusely infiltrating gliomas, depending on their proliferation and genetic landscape (DeWitt et al. 2017). Gliomagenesis is accompanied by genetic alteration, chromosome instability, and unregulated growth factor signaling pathways. Also, Genome sequencing uncovered aberrant epigenomes characterized by altered DNA methylation and histone modifications that are present across several adult and pediatric brain tumors.

	Glioma tumors	Clinical features	Grade
Gliomas with circumscribed growth	○ Pilocytic Astrocytoma ○ subependymal giant cell astrocytoma (SEGA)	Low proliferative	I
Gliomas with diffusely infiltrative growth	○ Diffuse Astrocytoma ○ Oligodendroglioma ○ Oligoastrocytoma	Infiltrative and recur	II
	○ Anaplastic Astrocytoma ○ Anaplastic oligodendroglioma ○ Anaplastic oligoastrocytoma	Malignant High mitotic activity Nuclear atypia	III
	○ Glioblastoma ○ Diffuse midline glioma, H3 K27M-mutant	The most frequent and malignant. Extensive necrosis and rapid clonal evolution.	IV

Table 1. Gliomas classification

1.1.1 Glioblastoma Multiform

Glioblastoma multiform (GBM) is the most common and aggressive malignant *de novo* primary brain tumor. GBMs occur mostly in patients over 50 years old, however, they can also be manifested in children and young adults. The latter is genetically different from adult glioblastoma. Molecular profiling has improved on the understanding of glioma pathogenesis. Using a comprehensive approach, diffuse gliomas are grouped based on their IDH (Isocitrate dehydrogenase) mutation profile (**Table 2**) (Lapointe et al. 2018; Chen et al. 2017a; Yan et al. 2009). There are two forms of IDH: cytosolic IDH1 and mitochondrial IDH2. IDH1/2 convert isocitrate to α-Ketoglutarate (α-KG), coupled with reduction of $NADP^+$. However, in IDH1/2 mutant glioma, α-KG is catalyzed to R(-)-2-hydroxyglutarate (2-HG) in a NADPH-dependent manner, thereby causing oxidative stress, histone methylation, and hyper-methylation of several CpG islands that are linked to a better prognosis in this type of tumor.

Most glioblastoma that occur in patients over 50 years old represent two distinct subgroups: the mesenchymal and the receptor tyrosine kinase (RTK) classes (Stommel et al. 2007). The

latter is characterized by higher amplification of the growth factor receptor (EGFR) and its active variant III (EGFRv III), which in turn promotes the tumor growth (Brennan et al. 2013). This feature makes EGFR a good target for immunotherapy. Moreover, a high level of the DNA repair protein O6-methylghuanine DNA methyltransferase (MGMT) in glioblastoma leads to temozolomide treatment resistance (Melguizo et al. 2012). Therefore, hyper-methylation of a CpG-rich region of MGMT promoter inhibits DNA repair in tumor cells treated with alkylating chemotherapies, which will be of benefit for glioblastoma patients (Esteller et al. 2000; Hegi et al. 2005).

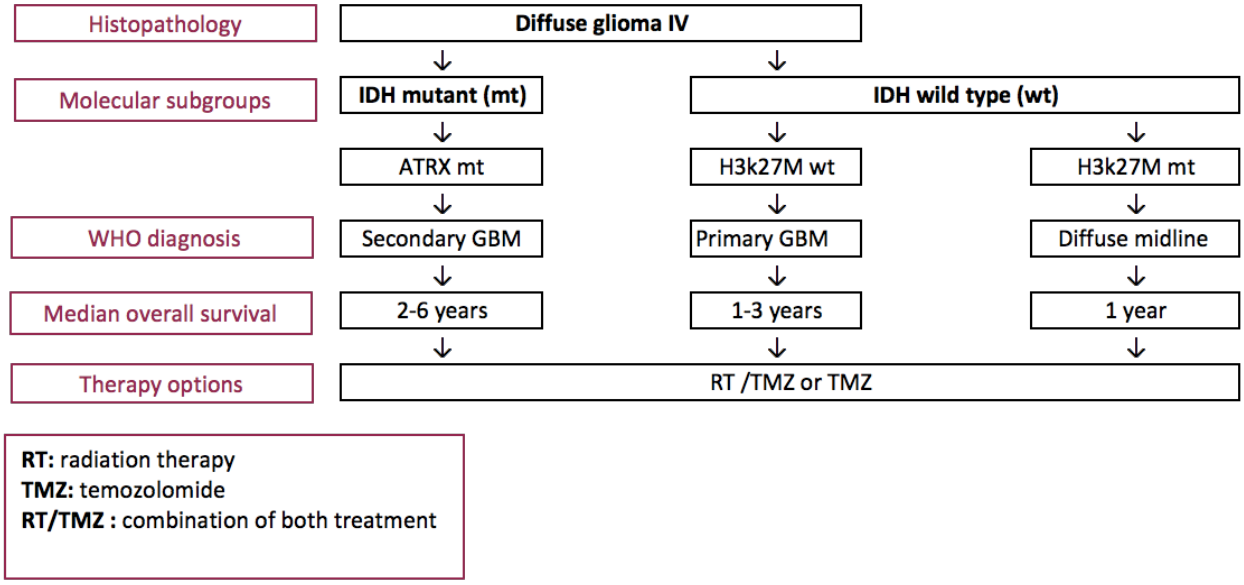

Table 2. Diffuse gliomas grade VI subgroups

1.1.2 Diffuse Intrisic Pontine Glioma

Brainstem gliomas represent 10–20% of pediatric brain tumors (Monje et al. 2011). They are clustered into diffuse intrinsic pontine gliomas (DIPG) that arise mainly in the ventral pons, and low-grade gliomas, which occur in the dorsal pons or midbrain. DIPG are extremely infiltrative and aggressive as compared to the low-grade ones that have a markedly better survival course (Wong et al. 1999; Buckner 2003). Notably, the compact structure of the brainstem is a major limitation in the penetration of the BBB in DIPG treatment. Thus, irradiation combined with chemo-radio therapy remain the standard treatment for DIPG (Gwak and Park 2017).

Childhood brain tumors can carry mutations in the genes, encoding the histone 3 variants H3.3 (*H3F3A*) and H3.1 (*HIST1H3B* and *HIST1H3C*), which lead respectively to the substitution of lysine (K) to methionine (M) at position 27 in the histone 3 tail (H3K27M)

and the subsequent glycine to arginine or valine at position 34 (G34R/V) (Jones et al. 2017). These mutations have not yet been found in elderly patients with glioblastoma.

Clinical diagnosis reveals that DIPGs have a very bad prognosis and they are specific and distinct from other high-grade gliomas. Furthermore, H3K27M mutation represents a major hallmark of DIPG tumors that drives DNA hypo-methylation and reduced trimethylation of histone H3 at lysine 27 (H3K27me3) are major hallmark of DIPG tumors (Chan et al. 2013).

It has been reported that H3K27M mutation drives DIPG oncogenesis by repressing the catalytic activity of the polycomb repressive group (PRC2) due to an increased binding affinity of the mutant histone to the PRC2 catalytic subunit, EZH2 (Mohammad et al. 2017). In counterpart, according to another study, EZH2 and its enzymatic product H3K27me3 are not recruited in chromatin sites enriched with H3K27M (Piunti et al. 2017). Therefore, the functional outcome of H3K27M remains incomplete and further investigations could advance our understanding of epigenetic mechanisms required for DIPG proliferation.

Advances in molecular biology have defined a new era in the epigenetic field, which holds promise for possible therapies of DIPG (Hashizume 2017). Different immune therapies for DIPG are being explored, including tumor vaccine strategies and the blockade of programmed cell death protein 1 (PD-1). Furthermore, pharmacological inhibition of the histone demethylase Jumonji domain containing 3 (JMJD3) or the methyltransferase enhancer of zeste homolog 2 (EZH2) reduces tumor growth and invasion in DIPG (Hashizume et al. 2014; Ridler 2017; Mohammad et al. 2017).

1.1.3 Glioma Models

Several laboratories use *in vitro* drug testing in order to develop potential targeted therapies for DIPG and glioblastoma. Usually, glioma cells are cultured as a monolayer with fetal bovine serum (FBS), but recently, neurospheres derived from the three-dimensional serum-free cell culture represent a powerful tool for *in vitro* drug screening. These neurospheres mimic the original tumor better because they are enriched with brain tumor stem cells (BTSC).

Gliomas were extensively studied in the cell culture system; however, this approach is not good enough to model the complexity of the *in vivo* tumor microenvironment as well as the angiogenesis and metastasis. Therefore, several approaches have been used to generate the glioma animal model. Glioma models were chemically induced by DNA alkylating agents and used to establish cell lines such as C6, 9L, and GL261 (Stylli et al. 2015). These established cell lines were transplanted into a syngeneic immunocompetent mouse in order to study the cross-talk between the tumor and its microenvironment (Benda et al. 1968) (Huszthy et al. 2012).

Similar to glioblastoma, DIPG rodent models are generated through the intracranial injection of rat glioma cell lines such as F98, 9L, and C6 into the brainstem of immunocompromised neonatal rats, leading to the formation of brainstem tumors. The human glioma xenograft models are established by the transplantation of cultured human monolayer cell lines that are derived from biopsy material (Shapiro et al. 1979). Human DIPG xenograft models are established by direct inoculation of either dissociated neurospheres or patient-derived DIPG cells in order to develop pontine DIPG-like tumors (Misuraca et al. 2016). The xenograft model is very useful to validate preclinical studies (Horten et al. 1981).

Genetically engineered immunocompetent mouse models also exist, and are very convenient to understand glioma development. These models are generated by specific genomic manipulation that can be either transgenic or loss of function (Fomchenko and Holland 2006).

1.1.4 Tumor Microenvironment in Glioma

Cancer research has been focused on neoplastic cells for many decades, but their influence on the tumor microenvironment has not had the same level of attention. Interactions between cancer cells and their stroma is vital and may disrupt the surveillance of the immune system for tumor survival and progression. The interaction between the microenvironment and the cancer cell was first proposed by a 19th century English surgeon, Stephen Paget, with his "seed and soil" hypothesis. He argued that cancer cells ("the seeds") selectively metastasize to neoplastic-promoting niches ("soil") (Langley and Fidler 2011; 2018). After "seeding", cancer cells actively corrupt their microenvironment by releasing growth factors, chemo-attractants, and cytokines. The tumor microenvironment is also clinically relevant, as it can affect patient prognosis and potentially confer drug resistance, resulting in potential relapse and metastasis.

A dynamic cellular microenvironment shapes and supports a glioma tumor. Indeed, glioma-secreted factors like cytokines and chemokines in order to attract and recruit astrocytes, pericytes, endothelial cells, and immune cells such as brain-resident microglia, peripheral macrophages, T cells, and myeloid-derived suppressor cells (MDSCs), which will maintain an immunosuppressive microenvironment (Gabrilovich et al.). Of note, the presence of infiltrating T cells and MDSCs in the glioma supports the hypothesis of a leaky BBB (Davies 2002; Jain et al. 2007). MDSCs in glioma are a heterogeneous population of immature precursor cells of macrophages, dendritic cells, and granulocytes (Gielen et al. 2015).These cells inhibit cytotoxic activity of natural killer cells and suppress adaptive immune response by promoting $CD4^+$ or $CD8^+$ T cell apoptosis. However, MDSC induces regulatory T cell (Treg) response, which is a potent inhibitor of effector T cells (Marvel and Gabrilovich 2015). In addition, glioma secrete B7-H1, TGF-β and interleukin 10(IL10) to inhibit anti-tumor T cell activation (**Figure 1**) (Wintterle et al. 2003; Perng and Lim 2015).

Glioma-associated microglia and macrophages are the predominant population in malignant gliomas. In brain tumors, bone marrow-derived macrophages exert the same role as microglia in promoting glioma progression (Gieryng et al. 2017b). Yet, the question remains about how to distinguish between microglia and infiltrating macrophages in glioma. The expression level of CD45 is commonly used to identify microglia ($CD45^{low}$) from tumor-associated macrophages ($CD45^{high}$) by flow cytometry in *in vivo* models of glioma tumors. However, it has been found that microglia are able to upregulate CD45 expression; thus, this marker does not accurately discriminate microglia and bone marrow-derived monocytes in human samples (Müller et al. 2015; Chen et al. 2017b). Recently, putative markers such as SALL1 and TMEM19 has been identified to separate microglia from peripherally derived macrophages in murine glioma models, (Bennett et al. 2016).

On the other hand, microglia and macrophages, infiltrating the tumor microenvironment of DIPG, are less inflammatory as compared to adult GBM. Also, lack of infiltrating lymphocytes leads to ineffective adaptive immune response in DIPG tumors (Lin et al. 2018).

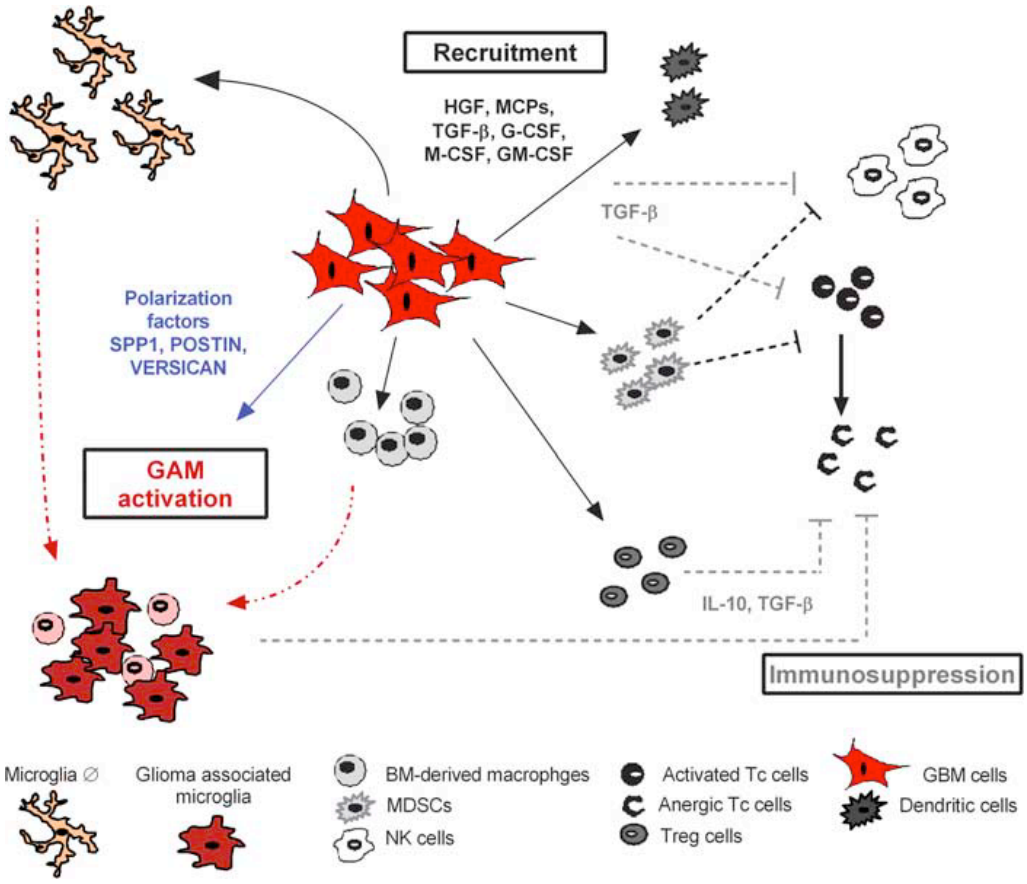

Figure 1. Illustration of the immune response in glioma microenvironment.
Adapted from (Gieryng et al. 2017b)

1.2 MICROGLIA

Microglia are the resident myeloid cells of the CNS. They constitute 10–20% of the non-neuronal cell population in the brain. Historically, microglia were first stained and characterized by Franz Nissl in 1880, and eventually termed microglia by Río-Hortega in 1919 (Ginhoux et al. 2013). They continually survey the brain and respond rapidly to stimuli and threats in their microenvironment. Indeed, they express surface receptors to detect changes due to brain damage or infections and migrate to the site of injury. Microglia are necessary for brain homeostasis, indeed, they have direct contact with other glia cells and neurons during brain development and neurogenesis. Several studies have been conducted in the last two decades to better understand the functional role of microglia in development, neurodegenerative diseases, and brain tumors. These innate immune cells of the brain can contribute to rather contrasting effect, promoting neuronal cell death or tumor cell progression. Their vital role in pathological events makes them a potential therapeutic target.

1.2.1 Microglia Ontogeny and Homeostasis

The origin of microglia has long been debated. They were first described as derived from hematopoietic stem cells in the bone marrow (Ginhoux et al. 2013). Notably, recent fluorescence-activated cell sorting approaches in mice have identified that microglia cells originate from myeloid progenitors in the yolk sac. They are $CD45^{low}$ and they express the transcription factor RUNX1 and the receptor tyrosine kinase c-Kit, also named CD117 during embryogenesis (Kierdorf and Prinz ; Salter and Stevens 2017). These pre-microglia migrate to the CNS via the circulatory system during development between day E8.5 and E9.5 before the establishment of the BBB. In the brain, microglia cells undergo different developmental steps, where they proliferate and acquire their ramified morphology. In the case of brain disease, the BBB is disrupted and other peripheral monocytes migrate and undergo differentiation in reaction to inflammation.

Since the microglia cell population arises during early embryogenesis, the adult microglial pool is able to renew itself in the CNS without the contribution of circulating monocytes. This finding has been supported in mice by bone marrow (BM) irradiation, chimerism (Ajami et al. 2007; Mildner et al. 2007), and cell lineage-tracing (Ginhoux et al. 2010). However, under pathological conditions, blood-derived and bone marrow-derived monocytes infiltrate and colonize the brain transiently and differentiate into cells that are phenotypically very similar to resident microglia (Ajami et al. 2011). Notably, CD45 antibody has been used intensively to distinguish microglia from macrophages. However, because microglia are able to upregulate CD45 expression in human glioma samples, CD45 does not accurately discriminate microglia and bone marrow-derived monocytes (Badie and Schartner 2000). This concept was supported by using irradiation chimeras with a protected head. This study

shows that most tumor associated macrophage/microglia (TAM) are actually intrinsic microglia (Müller et al. 2015).

The microglial population is different from other CNS cells and is considered the macrophages of the brain. Of note, gene expression and transcriptome profiling reveals distinct gene expression signatures of inactivated microglia compared to peripheral myeloid cells as well as other CNS cells (Mammana et al. 2018).

Balanced microglia development and proliferation depend on several molecular pathways. The myeloid transcription factor PU.1 is important for microglia development in the yolk sac. Its absence results in reduction of microglia density (Kierdorf et al. 2013). In addition, colony-stimulating factor 1 (CSF-1) is essential to maintain microglia homeostasis. Deficiency of CSF-1 or the ligand Interleukin 34 (IL34) leads to significant loss of microglia in embryonic and adult mouse brains (Askew et al. 2017).

1.2.2 Microglia Role within the CNS

Microglia have been extensively studied for their role in immunity and neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) as well as in tumor biology. Furthermore, there is strong evidence that highlights the important role of microglia during brain development. Microglia instruct the functions that are crucial for a healthy CNS, exemplified by programmed cell death and clearance of apoptotic newborn neurons, as well as pruning developing axons and synapses. Later in adulthood, they are responsible for regulating neuronal and synaptic plasticity. In rodent models, microglia can be stimulated through ATP release in their local environment, which is mediated by glutamate-receptor agonists. Interestingly, phagocytic microglia clean up the brain from accumulated apoptotic neurons that express "eat me" signals, like phosphatidylserine (PS) which recognized directly by microglial receptor the milk fat globule epidermal growth factor 8 (MFG-E8) (Hanayama et al. 2002).

Besides clearance of apoptotic cells, microglia promote neurogenesis and axonal wiring by producing neurotrophic factors, including the insulin-like growth factor 1(IGF-1) signaling pathway (Ueno et al. 2013) as well as Interleukin 1 beta (IL-1β) and the interferon gamma (IFN-γ) in the subventricular zone (Shigemoto-Mogami et al. 2014). They, also, regulate the number of neuronal precursor cells (NPCs) and in particular, Cunningham and colleagues demonstrate that the microglia activation state affects NPC survival (Cunningham et al. 2013). Indeed, microglia regulate synaptic circuits during early and late postnatal stages. In addition, they mediate synaptic pruning by removing unwanted synapses and promoting the generation of new ones (Wake et al. 2009; Paolicelli et al. 2011; Schafer et al. 2012;

Tremblay et al. 2010). Therefore, microglia functions in this context depend on the molecular signaling pathway and biological state.

1.2.3 Microglia Plasticity

Microglia are multitasking cells that survey brain function, under physiological and pathological conditions. They are very plastic and constitute a group of cells with diverse morphologies and biological activities, rather than a uniform cell population (Nimmerjahn et al. 2005). The cross-talk between microglia and their microenvironment results in change in microglia phenotype form ramified to amoeboid (Tam and Ma 2014) and accompanied by polarization to specialized functions in response to environmental cues (Hanisch 2002). Based on macrophage classification, the best characterized microglia activation phenotypes are the classical M1 or the alternative M2 activation. Microglia are activated toward the M1 phenotype in response to pathogens and is induced by interferon γ (IFN-γ) or lipopolysaccharides (LPS), resulting in the release of different inflammatory mediators like interleukines IL-1β, IL6, and nitric oxide (NO). Contrarily, upon stimulation of IL4 or IL13, microglia polarize toward the M2 phenotype, which is characterized by production of anti-inflammatory cytokines and arginase 1(Arg1), which is needed for tissue remodeling.

Advances in new technologies have defined a limitation of the M1/M2 classification concept for microglia/macrophage polarization that doesn't reflect the complexity of microglia/ macrophage activation and functions (Ransohoff 2016). Indeed, according to a recent study, tumor-associated microglia cannot be characterized by the M2 phenotype and its subtypes (Nayak et al. 2014; Szulzewsky et al. 2015).

Improved isolation method of microglia combined with genome-wide transcription analysis and chromatin sequencing approaches revealed that microglia harbor different transcription and epigenetic signatures depending of their specific functions which confirm that their role and location will shape their chromatin and genome. It is known that epigenetic patterns play crucial role in stem cell reprogramming and cellular differentiation. Like stem cells, microglia are very sensitive to changes in their microenvironment and they undergo cellular reprogramming in order to differentiate toward various phenotypes and fulfill their role in normal brain, but also in the context of diseases (Cheray and Joseph 2018). Thus, microglia cells acquire specific chromatin modifications to obtain their unique molecular signature and plasticity

Consequently, microglia polarization is a dynamic and complex process that depends on activation of different intracellular signaling pathways (Li and Graeber 2012; Nayak et al. 2014). There is strong evidence that microglia identity and activation is mainly the result of changes in gene expression patterns that rely on the transcriptional factor network and the chromatin landscape (**Figure 2**) (Boche et al. 2013; Sousa et al. 2018). Thus, it is important to identify molecular and epigenetic mechanisms that underlie microglia plasticity.

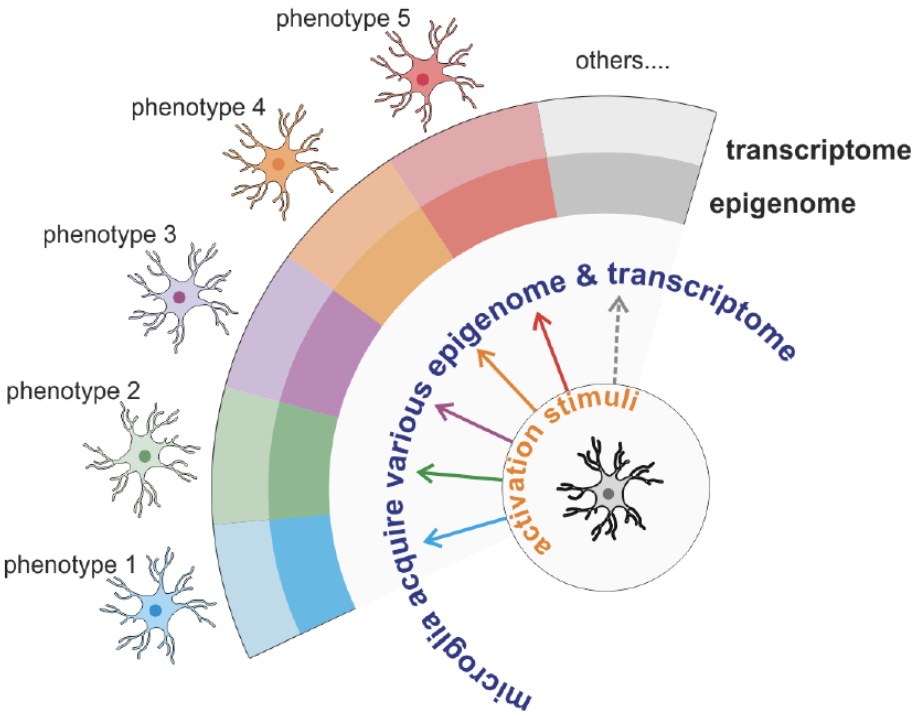

Figure 2. Microglia plasticity is associated with transcription and epigenetic changes.
Adapted and modified from (Cheray and Joseph 2018)

1.2.4 Tumor-Associated Microglia

Glioma cells release soluble factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF), which attract microglia in large numbers as the absence of GM-CSF decreases microglia invasion in organotypic brain slices and reduces intracranial glioma growth *in vivo* (Sielska et al. 2013). Hence, microglia density correlates with the malignancy of the glioma. Tumor associated microglia play an active part in promoting the invasive and proliferative features of glioblastoma. In fact, the pro-inflammatory cells switch to immunosuppressive microglia cells in the course of glioblastoma (Wei et al. 2013).

Glioma cells recruit and exploit microglia for their expansion. It has been reported that microglia cells and microglia secreted factors contribute to GL261 gliomas motility, however, endothelial cells and oligodendroglia have a moderate effect on glioma migration ability (Bettinger et al. 2002). In a similar study, deletion of endogenous microglia by treating organotypic brain slice cultures with liposomes carrying clodronate, results in reduced glioma invasiveness (Markovic et al. 2005).

Indeed, glioma attract microglia via monocyte chemoattractant protein-1 (MCP-1), also known as CCL2. Thereafter, glioma stimulates the release of IL6 from microglia by acting on the CCL2 receptor named CCR2 of microglia, which in turn promotes glioma invasiveness

(Saederup et al. 2010; Zhang et al. 2012). In addition, microglia promote glioma proliferation and invasion through activation of matrix metalloproteases (MMPs) as well as the release of epidermal growth factor (EGF) (Coniglio et al. 2012). For instance, overexpression of TGF-β in microglia will activate pro-MMP2 production in gliomas. Subsequently, gliomas release a chondroitin-sulfate proteoglycan named versican, which triggers the release of MMP9 and MMP14 (MT1-MMP) from microglia cells by activating microglial TLR2 and p38-MAP-kinase signaling. MMP14 cleaves pro-MMP2 produced by glioma into active MMP2, leading to degradation of the extracellular matrix (ECM) (**Figure 3**) (Markovic et al. 2009). Glioma stem cells (GSCs) use periostin (POSTN), also known as osteoblast-specific factor ECM protein, to attract microglia through the integrin receptor αvβ3. A report demonstrated that released factors from GSCs reduce microglia phagocytosis and stimulate the production of IL10 and TGF-β in microglial cells (Wu et al. 2010). However, it has been reported that naïve microglia can reduce tumor growth by inhibiting GSC sphere formation (Sarkar et al. 2013).

It is clear that microglia exhibit a considerable degree of plasticity in the glioma microenvironment, where gliomas are considered to drive microglia cellular reprogramming through chromatin modifications mediated by epigenetic regulators.

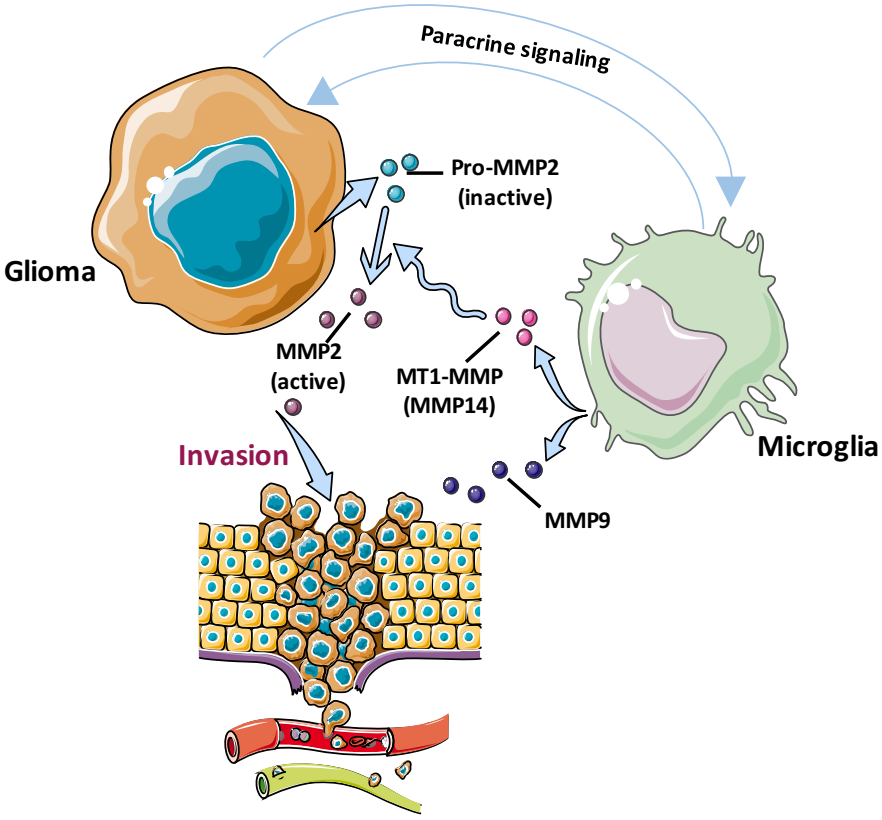

Figure 3. Microglia glioma cross-talk

1.3 EPIGENETICS

Epigenetics refers to modifications in gene expression without alterations in the genetic code (Berger et al. 2009). The epigenetic landscape is established during embryogenesis and defines the cell identity even after division. It helps us to better understand cell fate decision and the functional outcome to subsequent stimuli. Therefore, epigenetic signatures are considered traditionally stable and are mediated by posttranslational modifications of histone and DNA-binding proteins as well as by hydroxyl-methylation of CpG DNA motifs, nucleosome remodeling, and noncoding RNA (Bird 2007). Recently, it has become clear that these modifications can change within a matter of minutes in response to polarization stimuli and will define the chromatin accessibility for protein binding, such as signaling transcription factors and their localization in the genome.

Epigenetic modifications are considered a potential driver or inhibitor of tumor growth that is dramatically dictated by signaling alteration in their microenvironment (Mack et al. 2015). Similarly, they play an important role in sculpting the specificity of microglia polarization in response to different stimuli and is crucial for initial activation (Cheray and Joseph 2018).

1.3.1 Chromatin Architecture and Histone Modifications

In eukaryotic cells, the histone octamer consists of two units of the four core histone proteins H2A, H2B, H3, and H4. The positive charge given to the histone tail by the lysine assures the packaging of the long DNA molecules by opposing its negative charge. Originally, histones were considered as a static scaffold for DNA packaging (Luger et al. 1997). Indeed, they are able to wrap 147 base pair units of double-stranded DNA, forming a compact unit known as nucleosomes.

Histones were shown to be dynamic proteins undergoing posttranslational modification, involving phosphorylation, ubiquitination, methylation, and acetylation, which are written and erased by histone modifier enzymes. For instance, modification of the histone amino acid residues dictates the histone code pattern recognized by histone readers and will facilitate the recruitment of transcriptional coactivators or corepressors. A key concept is that chromatin accessibility can be remodeled by the balance between the repressive and positive histone modifications. Consequently, chromatin can condense, leading to transcriptional repression, or can be open for transcription-factors binding, which will promote gene expression (Cosgrove et al. 2004). The functional implication of these modifications is complex and depends on which residue they are located in the gene locus [the promoter or the gene body]

(Schneider and Grosschedl 2007). Methylation and acetylation of histones are the predominant epigenetic modifications. Histone methylation can act as a transcriptional repressor or activator. Histone residues are methylated by histone methyltransferases (HMTs) and demethylated by histone demethylases (HDMs). Besides, histones are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs) (Allis et al. 2007). In particular, lysine acetylation is associated with an open chromatin structure that promotes transcriptional activation (**Figure 4**) (Koch et al. 2007).

For instance, histone acetylation regulates the kinetics of gene induction for inflammatory response. Concordantly, it has been shown that HDAC inhibitors repress induction of various inflammatory genes (Blanchard et al. 2002) and have been used intensively to reduce LPS-induced microglia inflammatory activation *in vitro* (Durham et al. 2017). Likewise, a low level of histone acetylation, such as acetylation of lysine 9 on histone H3 (H3K9ac) correlates with reduced efficiency in induced reprogramming of nuclei from fibroblast, and HDACs inhibitors can improve the reprogramming efficiency (Hezroni et al. 2011).

1.3.2 Histone H4 Lysine 16 Acetylation

H4K16 acetylation plays an important role in transcriptional activation and modulation of chromatin structure. It is mainly regulated by the opposing effect of the HAT KAT8/MYST1/hMOF (human orthologue of the *Drosophila melanogaster* gene males absent on the first) and the NAD – dependent HDAC Sirtuin 1 (SIRT1).

The amount of H4K16ac in the nucleus depends on the cell type and environmental cues (Horikoshi et al. 2013). For instance, accumulation of H4K16 acetylation close to the transcriptional start site (TSS) of a specific gene will displace the nucleosome, resulting in an open chromatin structure, and acts as a binding platform for transcriptional factors and RNA polymerase complex in order to initiate the transcription (Schones et al. 2008). Alteration in the mark H4K16ac could result in genomic instability and aberrant gene expression (Li et al. 2010; Fullgrabe et al. 2011). Therefore, deregulation of this specific modification is linked to various diseases, including cancer (Gorisch et al. 2005; Hajji et al. 2010).

MOF is an acetyltransferase highly conserved among eukaryotes, and is a member of the MYST family (from the founding members: MOZ, Ybf2 / Sas3, Sas2, and Tip60). It was first identified in *Drosophila*, and is one of the key components of the dosage compensation complex or MSL (male specific lethal complex), which is required to balance gene expression between the sexes (Rea et al. 2007).

1.3.3 H4K16ac Remodelers

MOF is of particular interest as it displays quite diverse roles in various nuclear processes. It is responsible for acetylating H4K16 and non-histone proteins. Indeed, it is implicated in transcriptional regulation as well as in DNA damage, DNA repair, and cell growth by activating the protein kinase ATM (ataxia – telangiectasia mutated) and apoptosis by acetylating p53 (Mellert and McMahon 2009; Lu et al. 2011).

Most HATs target different types of histone residues; however, hMOF enzymatic activity has an extraordinary specificity for K16 at histone H4. Moreover, hMOF knockdown results in decrease or complete loss of H4K16ac. Therefore, any process mediated through H4K16ac can potentially be influenced by hMOF. Recent observations suggest that hMOF and H4K16ac may be involved in tumorgenesis (Rea et al. 2007; Zhao et al. 2013).

Sirtuins are highly evolutionarily conserved enzymes, which were first discovered in *Saccharomyces Cerevisiae* and named silence information regulators (SIR2). There are seven SIR2 mammalian sirtuins, termed SIRT1 through SIRT7 (Donmez 2012). The human protein SIRT1 has different domains, including nuclear localization signals (NLS), nuclear export signals (NES), essential for SIRT1 activity, and an enzymatic core. Besides, SIRT1 deacetylates both histones and non-histone proteins by hydrolyzing NAD+ and transferring the bounded acetyl group to an ADP- ribosyl group (Revollo and Li 2013). Indeed, hMOF autoacetylation changes the surface charge of the protein and alters its binding to the nucleosome. Thereby, SIRT1 deacetylates hMOF and promotes its recruitment on the chromatin leading to upregulation of H4K16ac (Lu et al. 2011).

SIRT1 is highly expressed during embryogenesis and in the adult mouse brain. SIRT1 participates in various cellular functions in distinct subcellular locations that may change during embryonic development and in response to biological stimuli (Hisahara et al. 2008). Therefore, its subcellular localization must affect its function.

SIRT1 has been extensively investigated in cancer and neurodegeneration (Donmez 2012). Clearly, small molecule activators or inhibitors of SIRT1 can prevent or treat age-related diseases (Outeiro et al. 2008; Zhang et al. 2011). Remarkably, SIRT1 has protective effect in neuronal toxicity (Araki et al. 2004; Anekonda and Reddy 2006) and prevents toxicity of microglia-dependent plaque deposition of amyloid B (AB) in AD by inhibiting the nuclear factor NF-κB signaling. SIRT1 interacts with the RelA/P65 subunit of NF-KB and inhibits transcription by deacetylating RelA/P65 at lysine 310 (Gan and Mucke 2008; Yang et al. 2012). Moreover, the SIRT1-p53-caspase 3-dependent apoptotic pathway is involved in PD. For instance, decrease of SIRT1 activity in microglia leads to high levels of TNF-α and IL6.

These microglia-derived factors will inhibit p53 deacetylation by reducing SIRT1 expression, and promote apoptosis in neurons (Ye et al. 2013). Collectively, H4K16ac and its regulators are implicated in various diseases, which may be potential targets for drug development.

1.3.4 Histone H3 Lysine 27 Tri-methylation (H3K27me3)

H3K27me3 is a hallmark of repressed transcription while acetylation of H3K27 is tightly associated with an active gene promoter (Tie et al. 2009). H3K27me3 is involved in complex biological processes, including cellular differentiation (Boyer et al. 2006; Bracken et al. 2006) inactivation of chromosome X (Rougeulle et al. 2004), and disease development. Trimethylated H3K27 is studied more than the mono- and di-methylation of lysine 27 at H3. H3K27me2 has the same distribution as H3K27me3; they act in opposition to H3K27me1 and H3K4me3 (Barski et al. 2007).

Tri-methylation of H3K27 is catalyzed only by EZH2, which is the catalytic subunit of PRC2. The polycomb group (PcG) proteins are implicated in the silencing of Hox gene expression as identified first in *Drosophila* (Lewis, 1978). These proteins play a key role in the maintenance of cell-type identity, differentiation, and disease by establishing themselves in a repressive chromatin. There are two polycomb repressive complexes (PRC) known as PRC1 and PRC2.

PRC2 binds to the chromatin and mediates H3K27 tri-methylation through its catalytic subunit, EZH2. H3K27me3 is then recognized by PRC1, leading to chromatin compaction and RNAPII pausing (Francis et al. 2004). EZH2 also acts as a binding platform for DNA methyltransferases at repressed promoters, thus linking histone methylation to DNA methylation (Vire et al. 2006). In various cancers, both PRC1 and EZH2 are upregulated (Aloia et al. 2013).

The ubiquitously transcribed tetratricopeptide repeat X (UTX) and the Jumonji domain containing 3 (JMJD3 known also as KDM6B) were first described in 2007 as histone demethylases, which contain the Jumonji C (JmjC) catalytic domain (Agger et al. 2007). They have an antagonist function for the EZH2 that regulates the balance of H3K27me3 level. Both enzymes are implicated in gene expression by maintaining an open chromatin state, and play a significant role in chromatin remodeling (Svotelis et al. 2011; Kooistra and Helin 2012). It has been demonstrated in macrophages using chromatin immunoprecipitation coupled with next generation sequencing (ChIP-Seq) data that the UTX/JMJD3 complex is enriched in H3K4me3 loci associated with active genes (De Santa et al. 2009). Alterations in

H3 lysine 27 demethylases lead to unbalanced gene expression. For instance, in mouse, loss of JMJD3 or UTX results in embryonic lethality. Moreover, JMJD3 and UTX are required for T cell subset development and neurogenesis (Arcipowski et al. 2016). JMJD3 and UTX have been intensively investigated for their critical functions in cellular reprogramming and cancer, and are considered potential therapeutic targets in many diseases (Agger et al. 2007; Ezponda et al. 2017).

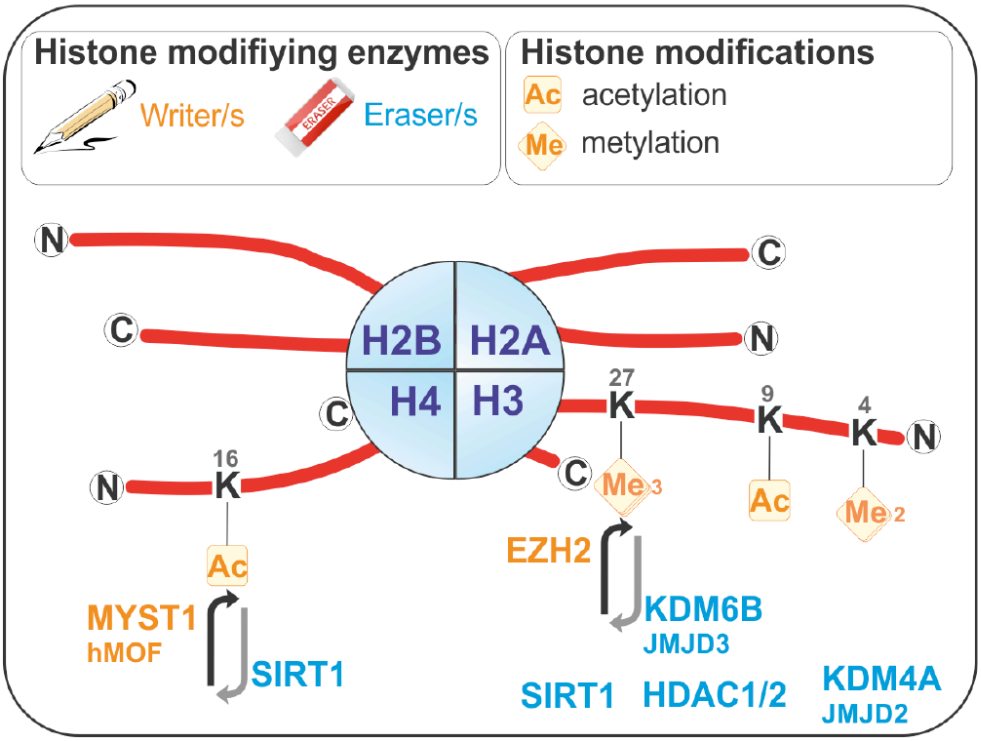

Figure 4. Post-translational modifications on histone tails.
Adapted from (Cheray and Joseph 2018)

1.4 NITRIC OXIDE AND CASPASE 3

Nitric oxide (NO) in mammalian cells is produced via conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) (Lee et al. 2005). NOS2, one of the three NOS isoforms, has a vital function in immune response and cancer. Therefore, it is induced by inflammatory stimuli like LPS and TNFα, causing the production of NO in large amounts. The NO signaling pathway mediates posttranslational modifications like S-nitrosylation of cysteine residues on proteins that are involved in cellular functions. Moreover, massive production of NO was also observed in several cancers, including high-grade glioma (Cobbs et al. 1995; Choudhari et al. 2013). Thus, NO can promote angiogenesis, invasion and tumor progression (Ying and Hofseth 2007). Highly conserved caspases (cysteine dependent aspartate directed proteases) are a family of proteases that cleave their substrate at aspartate residues. They are expressed as inactive pro-form (pro-caspase) that need to be dimerized and cleaved into an active caspase. They are involved in different biological processes and are grouped based on their function. For cell apoptosis, caspase -3, -6, and -7 are the executioner caspases, while caspase -2, -8 -9, and -10 represent the initiators. However, caspase -1, -4, -5, and -12 are involved in inflammation (McIlwain et al. 2013).

For many years, caspase-3 was associated only with cell death. However, an elegant study conducted by Burguillos and colleagues reveals a new role for executioner caspases in the microglia activation process. In this study, they discovered that active caspase-3 was implicated in microglia activation through the Toll-like receptor 4 (TLR4) upon stimulation by lipopolysaccharide (LPS), but without microglia cell death. Indeed, caspase-8 activates caspase-3, which in turn will cleave protein kinase c-δ (PKC-δ) leading to activation of IκB Kinase (IKK) complex that will cause release of NF-κB. Thereafter, NF-κB translocates to the nucleus and promotes gene expression of pro-inflammatory genes such as IL1β, NOS2, and TNFα. Thus, caspase-3 activation in microglia is linked to neurotoxicity. But why does caspase-3 not kill microglia cells? Activation of microglial caspase-3 through TLR signaling leads to upregulation of the cellular inhibitor of apoptosis proteins (cIAPs), which in turn will prevent further cleavage of caspase-3 subunit P19 to fully active P17 (Kavanagh et al. 2014). The catalytic site of caspase-3 is cyctein (Cys) 163. It can be inactivated by the S-nitrosylation posttranslational modification conducted by NO that is added to cysteine thiol, resulting in protein S-nitrosylation. Conversely, S-nitrosylation is reversible; thus, caspase-3 is denitrosylated by thioredoxin (Trx) (Benhar et al. 2008). Trx are highly conserved proteins (Lillig and Holmgren 2007) and exist in three forms: cytosolic (Trx1), mitochondrial (Trx2), and testis-specific (Trx3) (Jimenez et al. 2004). Trx proteins are able to transfer electrons from NAPDH to proteins via the NADPH-dependent enzyme thioredoxin reductase.

2. AIMS OF THE THESIS

Microglia are the innate immune cells of the CNS. They have an impressive level of plasticity that makes them able to adapt to changes in their microenvironment. Gliomas recruit microglia, which in turn promote their expansion and invasion. The general aim of my studies was to define the role for molecular mechanisms within microglia and glioma communication, and to decipher the epigenetic control in cellular reprogramming of microglia during the course of the disease.

Specific aims of the projects:

Paper I

To study the role of histone modifications, especially H4K16ac in microglia polarization toward tumor promoting phenotype.

Paper II

To investigate a potential involvement of H3K27me3 modifiers enzymes, EZH2 and JMJD3/UTX in the regulation of tumor associated microglia in the context of pHGG and DIPG.

Paper III

To explore how caspase 3 signaling pathway is implicated in the activation of glioma associated microglia.

3. RESULT AND DISCUSSION

Paper I. Glioma-induced SIRT1-dependent activation of hMOF histone H4 lysine 16 acetyltransferase in microglia promotes a tumor-supporting phenotype

In this study, we first measured and quantified the microglial global amount of H4K16ac in both *in vitro* and *in vivo* models upon glioma-induced microglia activation. To explore *in vitro* the effect of glioma stimuli on microglia, we performed a cell segregated co-culture approach that was used for all the co-culture experiments in this study. In this setting, microglia were seeded in the upper compartment, while we cultured glioma cell lines from different origins at the bottom of the dish. Thereby, microglia cells were stimulated through soluble factors in the medium originating from glioma cells.

To investigate microglia activation in response to glioma-released factors, gene expression profiling and functional assays were carried out in microglia. Of note, a recent study demonstrates that rat C6 glioma has a similar immune microenvironment to human glioblastomas (Gieryng et al. 2017a). We observed that C6 gliomas migrate further in the presence of BV2 microglia. Furthermore, we detected induction of a tumor-supporting phenotype in BV2 microglia. Using quantitative PCR (QPCR), we found that the mRNA expression of the inflammatory marker NOS2 was downregulated, whilst there was an upregulation of the tumor-supportive markers IL6, IL1β, CCL22, YM1, and MMP14 in BV2 cells upon segregated co-culture with the C6 glioma cell line for 4h (Fig. 1a). Notably, IL6 signaling maintains microglia tumor-supporting functions and promotes glioma malignancy by supporting glioma stem cell growth (Wang et al. 2009; Zhang et al. 2012)

Interestingly, in BV2 cells subject to co-culture with the C6 glioma cell line at 2h, we detected a transient decrease of H4K16ac global protein level, followed by a significant increase at 4h, 6h, and 24h without affecting microglial H4 levels (Fig. 1e-f; Supplementary Fig. 1a-b).

Likewise, we found a similar upregulation of H4K16ac in BV2 microglia segregated co-cultured with glioma cells of distinct origins, including the GL261 glioma cells and murine primary glioma tumor spheres (Supplementary Fig. 1c-f). Notably, H4K16ac is a hallmark of accessible chromatin and an important modulator of transcriptional activation. The balance of the H4K16 acetylation level is regulated by the histone acetyltransferase hMOF and the deacetylase SIRT1. For instance, SIRT1 is implicated in various biological processes. SIRT1 reduction leads to aging and memory deficit mediated by Tau through the upregulation of IL1β (Cho et al. 2015).

The microglial hMOF protein level was not found to be altered upon glioma co-culture conditions, suggesting that the observed increase of H4k16 acetylation level was not due to an aberrant protein expression level of the acetyltransferase hMOF (Fig. 3c). Conversely, the observed upregulation of H4K16ac correlates with an accumulation of SIRT1 in the BV2 microglia nucleus upon exposure to glioma cells at 4h (Fig. 3e-g). However, the transient downregulation of the SIRT1 protein level was due to proteasomal degradation in response to glioma stimuli (Supplementary Fig. 3c). Indeed, the nucleo-cytoplasmic shuttling of SIRT1 is mediated by two nuclear localization signals and two nuclear export signals. SIRT1 exhibits various functions depending on subcellular compartments, where it can be affected by specific posttranslational modifications.

To validate the *in vitro* results, we used an established *in vivo* GL261 glioma model. This model has been used extensively to study the immune microenvironment of gliomas. This glioma model benefits our study because it has limited infiltration by peripheral monocytes/macrophages (Müller et al. 2015). Using confocal microscopy, the fluorescence intensity quantification of microglia in the brain tissue surrounding the gliomas at 1 and 2 weeks after transplantation revealed a high abundance of H4K16ac in microglia cells located inside the tumor mass, as compared to cells residing at a distance from the tumor (Fig. 2a-c and Supplementary Fig. 2). Likewise, we found that SIRT1 was highly expressed in microglia nuclei inside the tumor as compared to the non-tumor area (Fig. 4 and Supplementary Fig. 4).

This implies that the microglia protumoral phenotype in glioma involves SITR1 nuclear translocation, accompanied by a high level of H4K16ac in BV2 cells. This finding appears to counteract the intrinsic deacetylase activity of SIRT1 on H4K16ac.

At first, these observations may sound contradictory; however, it is important to note that SIRT1 can be both a negative direct and positive indirect modulator of the H4K16 acetylation level. Indeed, the deacetylase SIRT1 directly decrease the expression level of this histone posttranslational modification . However, autoacetylation of hMOF reduces its enzymatic activity against H4K16ac. The autoacetylated hMOF at its lysine residue Lys274, can be activated via deacetylation by SIRT1 and, in turn, induce H4K16 acetylation (Michan and Sinclair 2007; Zhang et al. 2011)

Based on these observations, we investigated SIRT1 and hMOF protein interactions by co-immunoprecipitation (coIP) and *in situ* proximity ligation assay (PLA) First, we found that SIRT1 and hMOF protein-protein interaction increased in microglia under segregated co-culture conditions with glioma cells for 4h (Fig. 5a-c). The increase of SIRT1 and hMOF

interaction in glioma-induced microglia correlates to a decrease in microglial basal acetylated hMOF upon co-culture for 4h, which was illustrated using anti-acetylated lysine

immunoprecipitated protein complexes and PLA (Fig. 5d-f). Finally, we observed significant hMOF recruitment by chromatin immunoprecipitation (ChIP), coupled with higher H4K16ac enrichment at the Il6, IL1β, Mmp14, Ccl22, and Chil3 promoter regions (Fig. 6a-j). However, SIRT1 was not recruited at these promoter regions in microglia cells upon glioma stimulation (Supplementary Fig. 5). These data strengthen our hypothesis that activation of microglia by glioma cells induces translocation of SIRT1 into the nucleus of microglia cells, which will deacetylate hMOF, leading to its recruitment and acetylation of H4K16 at the promoter of microglial tumor-supportive markers.

Our next step was to investigate the functional outcome of H4K16 acetylation level *per se* on microglia activation toward the pro-tumoral phenotype. To manipulate acetylation of H4K16, we took advantage of the intrinsic enzymatic effect of histone deacetylase SIRT1 or acetyltransferase hMOF on H4K16ac. BV2 cells were pretreated either with small interfering RNA (siRNA) against SIRT1 or EX527, a selective SIRT1 inhibitor that results in upregulation of H4K16 acetylation. Activating SIRT1 with SRT1720 reduces the acetylation of this histone mark. Therefore, the increase of H4K16ac in microglia promotes the migration ability of gliomas as compared to untreated (the control ones) BV2 microglia (Fig. 7c, e, and f). In contrast, downregulation of H4K16ac in microglia through activation of SIRT1 decreases glioma migration. In agreement, similar effects on glioma migration ability were observed when the H4K16 acetylation expression level decreased due to hMOF gene expression silencing in microglia (Fig. 7g-h).

Taken together, our data indicate that, in the context of glioma-induced microglia activation, microglial SIRT1 primarily mediates its effect via the deacetylation of hMOF that, in turn, will enhance the recruitment of this H4K16 histone acetyltransferase to the chromatin and, subsequently, transcriptional activation of specific microglia target genes. Next, we demonstrated that H4K16 acetylation balance mediated directly by the intrinsic activity of SIRT1 and hMOF affects microglia polarization toward the tumor-supporting phenotype in glioma biology (Fig. 8) (**Figure 5**).

Paper II. Inhibition of EZH2 or JMJD3 reduces microglial pro-tumoral activation in Diffuse Intrinsic Pontine Glioma

Similar to other gliomas, microglia constitute approximately one-third of the cellular mass of DIPG (Caretti et al. 2014). In DIPG and pediatric high-grade gliomas, the functional role of infiltrating pontine microglia as well as their molecular profile remain elusive. In this project, we aim to explore how DIPGs affect microglia activation.

Thus, we used an *in vitro* segregated co-culture setting, where we seed BV2 microglia cells in the upper compartment; a monolayer of primary human DIPG cell line SF8628 or pHGGs (pediatric high-grade glioma) of human cell line SF188 are cultured at the bottom of the dish. The microglia and glioma will exchange secreted factors in the medium.

To elucidate the activation profile of BV2 microglia in the context of DIPG and pHGGs, we used a functional assay and found that microglia promote both SF188 and SF8628 invasion ability (Fig. 1B-C). In addition, we analyzed NOS2 and IL6 gene expression upon 4h co-culture. In line with other gliomas, we observed a significant downregulation of NOS2 and an upregulation of IL6 in DIPG-induced microglia. Strikingly, pHGGS-induced microglia have a non-inflammatory profile marked by a strong decrease in NOS2 and unchangeable basal microglial IL6 expression level (Fig. 1D-E). This indicates that microglia display various activation states in response to gliomas of different origins.

Since DIPGs harbor a global loss in H3K27me3, we further investigated the impact of this histone mark on microglia activation in the context of DIPG and pHGG that have a steady H3K27me3 state. We quantified H3K27me3's total protein level by immunoblot in BV2 microglia under co-culture, with either DIPG or pHGG for 4h. We detected a significant reduction of H3K27me3, while H3 expression was not altered in both conditions (Fig. 2).

H3K27me3 levels are regulated by the histone demythelase KDM6 enzymes named JMJD3 and UTX that counteract the histone methyltransferase EZH2. We further investigated the histone modifying enzyme(s) involved in the regulation of H3K27 trimethylation observed in microglia upon their activation by glioma cells SF8628 and SF188 at 4h and observed a downregulation of microglial JMJD3 mRNA expression, while there is no significant alteration in the mRNA expression level of UTX and EZH2, except for a slight decrease of UTX gene expression in microglia co-cultured with SF8628 (Fig. 2E). Our data suggests that tumor-associated BV2 microglia in DIPG or pHGG is coupled with a downregulation of H3K27me3 and the demethylase JMJD3, which appear to be contradictory. Strikingly, ChIP assessments show that JMJD3 was recruited to genes enriched with the activation mark H3K4me3 rather than with H3K27me3 upon LPS treatment in macrophages *in vitro* and *in vivo* (**Figure 5**) (De Santa et al. 2009).

In fact, the discovery of KDM6 demethylases changed our views from the original perspective that H3K27 methylation was not catalytically reversible. Thus, JMJD3 and UTX modulate H3K27me3 dynamics. However, they have a limited impact on genome-wide H3K27me3 steady-state homeostasis. For instance, a dramatic loss of H3K27me3 has been shown in the promoter of HOX genes in mouse embryonic fibroblasts (MEFs) UTX /JMJD3-deficient. This observation suggests an additional mechanism for H3K27 demethylation that

remains unclear (Bosselut 2016). JMJD3 is an important regulator of microglia polarization. EZH2 gene expression was also found to rapidly upregulate in microglia, subject to LPS-mediated TLR4 stimulation (Das et al. 2017b). Interestingly, it has been reported that inhibition of JMJD3 or EZH2 small molecule inhibitors reduces tumor growth in DIPG *in vivo* and *in vitro*. Thus, our next step was to investigate the effect of those inhibitors on the functional outcome of microglia in the context of DIPG and pHGG biology. It is clear that pretreating BV2 microglia with JMJD3 inhibitor GSKJ1 has a negative effect on SF188 and SF8628 invasion (Fig 3C-D). Concordantly, increased intrinsic JMJD3 suppresses LPS-induced microglial pro-inflammatory activation and promotes alternative activation of M2 microglia (Tang et al. 2014; Das et al. 2017a).

Similarly, pretreating microglia with EZH2 inhibitor GSK343 leads to a potent decrease of the invasion ability of SF188 and SF8628 (Fig 3C-D). In contrast, the use of the same EZH2 inhibitor in SF8628 DIPG or the SF188 pHGG prior to co-culture with BV2 microglia was not able to suppress microglia tumor-supporting phenotype activation illustrated by NOS2 gene expression (Fig. 4B). This data indicates that microglia could influence the biology and treatment of DIPG or pHGG.

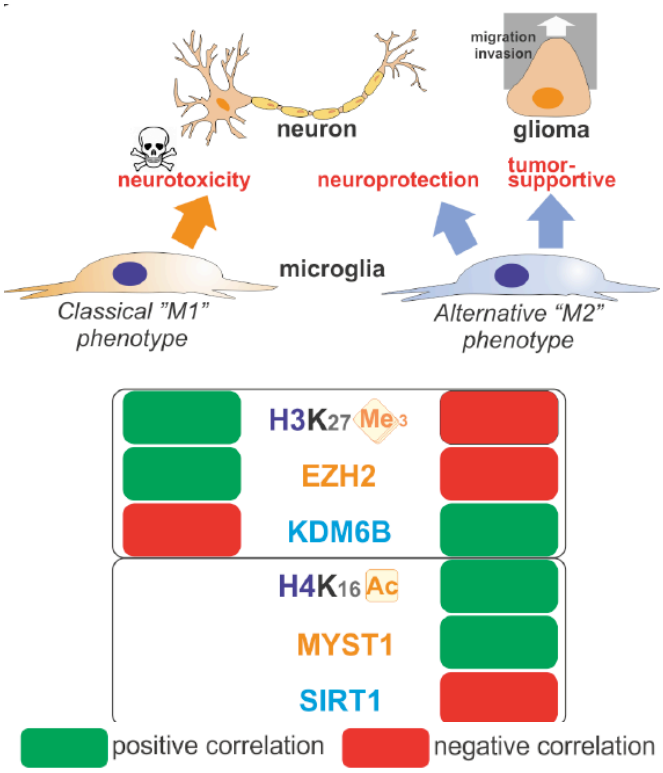

Figure 5. Histone modification associated with microglia polarization.
Adapted and modified from (Cheray and Joseph 2018)

Paper III. Glioma-induced inhibition of caspase-3 in microglia promotes a tumor-supportive phenotype

Previously, our lab reported a new role for executioner caspases in the microglia activation process. Microglia treated with LPS have high caspase-3-like activity, which induces microglial inflammatory response (Burguillos et al. 2011). Therefore, since caspase-3 is involved in the activation of microglia pro-inflammatory phenotype, we aimed to investigate whether the same signaling is occurring in microglia activation in the context of brain cancer.

We grew different microglia cells with glioma cells from distinct origins in a co-culture setting and quantified microglia caspase activity in response to glioma stimuli. We detected a decrease in microglial caspase-3 activity (Fig. 1a-g). Thus, glioma shut down caspase-3 activity in microglia. We observed a higher decrease of cleaved caspase-3 in Iba1 positive microglia inside the tumor as compared to microglia in the border reaching the tumor when we used an *in vivo* GL261 glioma model (Fig. 1h-j). Therefore, even *in vivo*, a glioma is able to repress caspase-3 activity in microglia cells. Next, we investigated the functional outcome of the low caspase-3 activity in microglia. Removal of capase-3 from microglia cells using siRNA leads to overexpression of the marker linked to the microglia tumor-supporting phenotype (IL6, CCL22, Ym1, and MMP14) while NOS2, a pro-inflammatory marker, decreased dramatically in microglia (Fig. 2a-d).

Moreover, we found that the caspase-3 knockdown in microglia helps gliomas to migrate and invade more as compared to microglia transfected with siRNA control (Fig. 2e). These data indicate that decrease of caspase-3 activity in microglia polarize them toward tumor-supportive phenotype.

Therefore, we defined the mechanism that gliomas use to induce the decrease of caspase-3-like activity in microglia. First, we examined the effect of the NOS2-derived NO exchanged between microglia and glioma on caspase-3 activity in microglia. We reported that an NOS2-specific inhibitor (1400W) prevents glioma induced-caspase-3 activity reduction in microglia (Fig 4a). Furthermore, using biotin-switch method and PLA techniques, we found that the active site of caspase-3 was S-nitrosylated at the cysteine residue in microglia cells after exposure to glioma cells (Fig 4b-c). For instance, the complete inhibition of NOS2 mRNA expression by siRNA in glioma or microglia led us to figure out that the NO produced and secreted by glioma and not microglia, causes S-nitrosylation of caspase-3 and thereby it becomes inactivated (Fig 4d-f). Finally, we revealed that the reduction of basal microglial caspase-3 activity and accumulation of SNO-procaspase-3 in the mitochondria in microglia is due to glioma-induced inhibition of thioredoxin-2 activity (Fig. 5c-d). Indeed, the knockdown

of Trx2 in microglia mimics the glioma effects on microglia regarding the level of SNO-procaspase-3 (Fig 5b).

Ultimately, we validated our previous findings *in vivo* by blocking the upstream signaling. Thus, removing NO from the GFP-GL261 glioma cells before injecting them into the mouse brain via NOS2 shRNA causes a decrease in tumor formation and a potent reduction of microglia recruitment to the tumor site (Fig. 6c-f). To gain further insight of the downstream signaling pathway involved in glioma cell-microglia communication, we generated a microglial caspase-3 knockout model. When we implanted GFP-GL261 in $Casp3^{flox/flox}CX3cr1^{CreERT2}$, we observed the development of a bigger tumor than the control counterparts after 1 and 2 weeks after tumor transplantation (Fig 6b-e).

In summary, we uncovered a novel key role for caspase-3 in the regulation of microglia activation. Gliomas recruit microglia and reprogram them by producing NOS2-derived NO, leading to accumulation of S-nitrosylated caspase-3 in microglial mitochondria due to glioma-induced inhibition of thioredoxin-2 activity. Therefore, gliomas inhibit microglial basal caspase-3 activity for their expansion and invasion (Shen et al. 2018) (**Figure 6**).

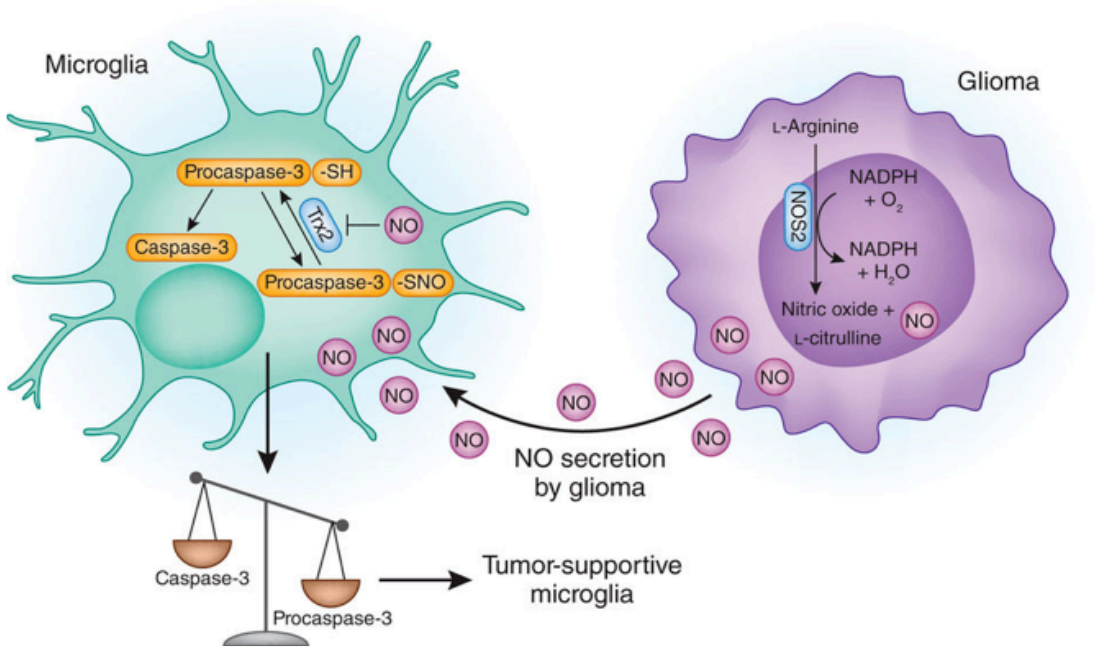

Figure 6. Caspase-3 regulate microglia tumor supporting phenotype in glioma.
Adapted from (Grauwet and Chiocca 2016)

4. CONCLUSION AND PERSECTIVES

Paper I:

During glioma-microglia cell communication, microglia undergo epigenetic reprogramming. We discovered that a glioma induces an increase of H4K16ac in microglia due to accumulation of SIRT1 in microglial nucleus, which in turn deacetylates hMOF, leading to its chromatin recruitment and upregulation of its enzymatic activity against H4K16ac at the promoter of specific microglia tumor-supporting genes. It is worth investigating the mechanisms that the glioma uses to induce nuclear translocation of microglial SIRT1; it could be due to oxidative stress generated by the glioma (Nasrin et al. 2009). In addition, the balance of the H4K16 acetylation level is critical for microglia plasticity in glioma biology.

Paper II:

In this study, we bring knowledge on how microglia cells are regulated in the H3K27M mutant DIPG context, wherein microglia activation toward the tumor-supporting phenotype is associated with a potent decrease of H3K27me3 and aberrant JMJD3 gene expression. Moreover, inhibiting histone enzymes EZH2 or JMJD3/UTX in microglia cells is reduced, which in turn decreases DIPG invasion. However, the investigations can be extended to decipher the signaling pathway that causes the global decrease of H3K27me3 in tumor-associated microglia and identify the downstream targets of JMJD3 and their involvement in microglia polarization. It is important to validate the effect of EZH2 and JMJD3/UTX inhibitors on DIPG microenvironment *in vivo*.

Paper III:

Caspase-3 may work as a rheostat, which controls the microglia cell fate in response to diverse stimuli, where low activity and reduced basal caspase-3 activity regulate, respectively, the pro-inflammatory and the tumor-supporting microglial activation states. It is of interest to identify mechanistically how low caspase-3 activity induces the polarization of microglia toward the tumor-supporting phenotype.

5. ACKNOWLEDGEMENTS

I want to acknowledge all the people that I have met or have been working with me during my PhD study at Karolinska Institutet.

First and foremost, I would like to express my sincere gratitude to my principal supervisor **Bertrand Joseph**. It is impossible to put into words how grateful I am for your guidance and moral support. You have opened the door to the world of academic research for me. Without your persistent patience of explaining questions to me and discussing all the details with me, I could not have done the thesis. You have always been encouraging and have made me believe that I might be doing a better job than I thought. The freedom you granted me for my work led to the number of results presented here.

I want to thank my co-supervisors **Nina Heldring** and **Ola Hermanson**, for good collaboration and for your time and your insightful advices. I appreciate your support.

It has been a great joy working with my collaborators **Klas Blomgren** and **Ahmed Osman** Thank you for your collaboration and exciting ideas. I feel fortunate to have had the chance to work with you. Thank you, **Ahmed**, for being always available to help and for your professional way in work.

I would like to thank all the former and current members of the pink group, without your help, this thesis would not have existed. **Popi** Thanks for caring and for always keeping the mood up. I appreciate your advice to me and your support. Your positive personality inspires me. **Mathilde**, you are an appreciated colleague, and I enjoyed working and sharing the office with you. Thanks for helping in answering my theoretical and practical questions. **Vassilis**, you have a great sense of humor. Thanks for the "party times" in the lab. Your help was invaluable in getting nice confocal microscopy images. Special thanks to **Olle**, your flow of ideas, motivation, and hard work are inspiring. You taught me many techniques, and you always had time to help me when I have a problem with experiments. **Kathleen**, thanks for being so kind and sharing your expertise in microglia. It was fun discussing science with you. Thanks, **Lara** for being a friendly colleague. I appreciate your help, and I wish you the best with your PhD project. **Julia**, your passion for science inspires me. Thanks for the great time we had at CCK and outside the work sometimes. I would also want to thank **Rachel** for working together with me and for being so dedicated.

My PhD life would not have been so much fun without **Patricia** and **Xianli**. You have been excellent friends and colleagues. Thanks for the great time we had at work and outside. It was tough, and you were always there. Thanks for given of your time, energy, and expertise to help me in research and life.

Also, former group members **Jens** and **Johanna** who gave valuable feedback, for which I would like to thank them. **Maria**, thanks for your warm-heart and your great help. Thanks also to **Hanzhao**, **Mimmi**, **Jeremy**, **Alex**, and **Agata**, I wish you the best in your future life.

I would like to thank all the members of **Boris Zhivotovsky's group**. I have enjoyed our group meeting and "fika."

It is impossible to mention all my colleagues at CCK you all made the department such an enjoyable place. Thanks to **Pedro**, **Rainer**, **Muyi**, **Ishani**, **Yuanyuan**, **Vicky**, **Ran**, **Ali**, **Dimitris** and **Aravindh** for being always friendly and helpful.Thanks to all the people I have met during my PhD studies at IMM. **Mizan,** it was always fun talking with you about science and general things in life. Thanks to **Imran**, **Jeremy** and **Aymen**, you have been supportive in many ways. I also want to thank my friends at Karolinska Institutet **Jelena** and **Evi**.

Erika Rindsjö, **Sören**, **Jenny Svedman, Lisa** and **Åsa Lycke** and all members of Karolinska Institutet's staff were very helpful with administrative issues. I am very grateful to you for making it so easy.

My gratitude also goes to my family and friends in Sweden. Special thanks to **Lars** for his warm and supportive friendship. We Have enjoyed dinners with you. Thanks for being always helpful. I would like to thank **Karin & Noamen**, **Sara & Sara**, **Lina & Aymen** and **Victoria & Hamadi** for the wonderful weekends and happy time we shared. Special thanks to **Khaled** who has continuously encouraged me whenever I felt frustrated.

My friends who are spread around the world: **Abyr**, **Douaa**, and **Souha** thanks for still caring despite my lack of courtesy when it comes to staying in touch. Your friendship is so valuable to me.

My family thanks for bringing even more joy to my short visits back home. You have always been in my heart!

Many thanks to my husband **Amine** for his endless love, patience, and support. Without you, my journey would have never been the same. I was continually amazed by your willingness to help me and to proofread pages of meaningless biology. You have experienced all of the ups and downs of my PhD and life. I love you! I want to thank **Amine's family** who has made my every single visit to Tunisia so exciting.

Last but not least, I want to thank **my parents** for their unconditional love and support, without whom I would never have enjoyed so many opportunities. I hope this will make them proud and make up for the missing time. This thesis is dedicated to my father's soul for his tough fight with cancer.

6. REFERENCES

2018. Stephen Paget and the 'seed and soil' theory of metastatic dissemination | SpringerLink.

Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K. 2007. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* **449**: 731-734.

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. 2011. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* **14**: 1142-1149.

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. 2007. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* **10**: 1538-1543.

Allis CD, Berger SL, Cote J, Dent S, Jenuwien T, Kouzarides T, Pillus L, Reinberg D, Shi Y, Shiekhattar R et al. 2007. New nomenclature for chromatin-modifying enzymes. in *Cell*, pp. 633-636, United States.

Aloia L, Di Stefano B, Di Croce L. 2013. Polycomb complexes in stem cells and embryonic development. *Development* **140**: 2525-2534.

Anekonda TS, Reddy PH. 2006. Neuronal protection by sirtuins in Alzheimer's disease. *J Neurochem* **96**: 305-313.

Araki T, Sasaki Y, Milbrandt J. 2004. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* **305**: 1010-1013.

Arcipowski KM, Martinez CA, Ntziachristos P. 2016. Histone demethylases in physiology and cancer: A tale of two enzymes, JMJD3 and UTX. *Curr Opin Genet Dev* **36**: 59-67.

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S et al. 2017. Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. *Cell Rep* **18**: 391-405.

Badie B, Schartner JM. 2000. Flow cytometric characterization of tumor-associated macrophages in experimental gliomas. *Neurosurgery* **46**: 957-961; discussion 961-952.

Ballabh P, Braun A, Nedergaard M. 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* **16**: 1-13.

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* **129**: 823-837.

Benda P, Lightbody J, Sato G, Levine L, Sweet W. 1968. Differentiated rat glial cell strain in tissue culture. *Science* **161**: 370-371.

Benhar M, Forrester MT, Hess DT, Stamler JS. 2008. Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. *Science* **320**: 1050-1054.

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A et al. 2016. New tools for studying microglia in the mouse and human CNS.

Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. 2009. An operational definition of epigenetics. *Genes Dev* **23**: 781-783.

Bettinger I, Thanos S, Paulus W. 2002. Microglia promote glioma migration. *Acta Neuropathol* **103**: 351-355.

Bird A. 2007. Perceptions of epigenetics. *Nature* **447**: 396-398.

Blanchard F, Kinzie E, Wang Y, Duplomb L, Godard A, Held WA, Asch BB, Baumann H. 2002. FR901228, an inhibitor of histone deacetylases, increases the cellular responsiveness to IL-6 type cytokines by enhancing the expression of receptor proteins. *Oncogene* **21**: 6264-6277.

Boche D, Perry VH, Nicoll JA. 2013. Review: activation patterns of microglia and their identification in the human brain. *Neuropathol Appl Neurobiol* **39**: 3-18.

Bosselut R. 2016. Pleiotropic functions of H3K27Me3 demethylases in immune cell differentiation. *Trends Immunol* **37**: 102-113.

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK et al. 2006. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**: 349-353.

Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. 2006. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev* **20**: 1123-1136.

Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH et al. 2013. The somatic genomic landscape of glioblastoma. *Cell* **155**: 462-477.

Buckner JC. 2003. Factors influencing survival in high-grade gliomas. *Semin Oncol* **30**: 10-14.

Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL et al. 2011. Caspase signalling controls microglia activation and neurotoxicity. *Nature* **472**: 319-324.

Caretti V, Sewing AC, Lagerweij T, Schellen P, Bugiani M, Jansen MH, van Vuurden DG, Navis AC, Horsman I, Vandertop WP et al. 2014. Human pontine glioma cells can induce murine tumors. *Acta Neuropathol* **127**: 897-909.

Chan KM, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, Gupta N, Mueller S, James CD, Jenkins R et al. 2013. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev* **27**: 985-990.

Chen R, Smith-Cohn M, Cohen AL, Colman H. 2017a. Glioma Subclassifications and Their Clinical Significance. *Neurotherapeutics* **14**: 284-297.

Chen Z, Feng X, Herting CJ, Garcia VA, Nie K, Pong WW, Rasmussen R, Dwivedi B, Seby S, Wolf SA et al. 2017b. Cellular and Molecular Identity of Tumor-Associated Macrophages in Glioblastoma. *Cancer Res* **77**: 2266-2278.

Cheray M, Joseph B. 2018. Epigenetics Control Microglia Plasticity. *Front Cell Neurosci* **12**.

Cho SH, Chen JA, Sayed F, Ward ME, Gao FY, Nguyen TA, Krabbe G, Sohn PD, Lo I, Minami S et al. 2015. SIRT1 Deficiency in Microglia Contributes to Cognitive Decline in Aging and Neurodegeneration via Epigenetic Regulation of IL-1 beta. *Journal of Neuroscience* **35**: 807-818.

Choudhari SK, Chaudhary M, Bagde S, Gadbail AR, Joshi V. 2013. Nitric oxide and cancer: a review. *World J Surg Oncol* **11**: 118.

Cobbs CS, Brenman JE, Aldape KD, Bredt DS, Israel MA. 1995. Expression of nitric oxide synthase in human central nervous system tumors. *Cancer Res* **55**: 727-730.

Coniglio SJ, Eugenin E, Dobrenis K, Stanley ER, West BL, Symons MH, Segall JE. 2012. Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling. *Mol Med* **18**: 519-527.

Cosgrove MS, Boeke JD, Wolberger C. 2004. Regulated nucleosome mobility and the histone code. *Nat Struct Mol Biol* **11**: 1037-1043.

Cunningham CL, Martinez-Cerdeno V, Noctor SC. 2013. Microglia regulate the number of neural precursor cells in the developing cerebral cortex. *J Neurosci* **33**: 4216-4233.

Das A, Arifuzzaman S, Yoon T, Kim SH, Chai JC, Lee YS, Jung KH, Chai YG. 2017a. RNA sequencing reveals resistance of TLR4 ligand-activated microglial cells to inflammation mediated by the selective jumonji H3K27 demethylase inhibitor. *Sci Rep* **7**: 6554.

-. 2017b. RNA sequencing reveals resistance of TLR4 ligand-activated microglial cells to inflammation mediated by the selective jumonji H3K27 demethylase inhibitor. *Scientific Reports* **7**: 6554.

Davies DC. 2002. Blood-brain barrier breakdown in septic encephalopathy and brain tumours. *J Anat* **200**: 639-646.

De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, Bucci G, Caganova M, Notarbartolo S, Casola S et al. 2009. Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *Embo j* **28**: 3341-3352.

DeWitt JC, Mock A, Louis DN. 2017. The 2016 WHO classification of central nervous system tumors: what neurologists need to know. *Curr Opin Neurol* **30**: 643-649.

Donmez G. 2012. The neurobiology of sirtuins and their role in neurodegeneration. *Trends Pharmacol Sci* **33**: 494-501.

Durham BS, Grigg R, Wood IC. 2017. Inhibition of histone deacetylase 1 or 2 reduces induced cytokine expression in microglia through a protein synthesis independent mechanism. *J Neurochem* **143**: 214-224.

Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. 2000. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* **343**: 1350-1354.

Ezponda T, Dupéré-Richer D, Will CM, Small EC, Varghese N, Patel T, Nabet B, Popovic R, Oyer J, Bulic M et al. 2017. UTX/KDM6A Loss Enhances the Malignant Phenotype of Multiple Myeloma and Sensitizes Cells to EZH2 inhibition. *Cell Rep* **21**: 628-640.

Fomchenko EI, Holland EC. 2006. Mouse models of brain tumors and their applications in preclinical trials. *Clin Cancer Res* **12**: 5288-5297.

Francis NJ, Kingston RE, Woodcock CL. 2004. Chromatin compaction by a polycomb group protein complex. *Science* **306**: 1574-1577.

Fullgrabe J, Kavanagh E, Joseph B. 2011. Histone onco-modifications. *Oncogene* **30**: 3391-3403.

Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* **12**: 253-268.

Gan L, Mucke L. 2008. Paths of convergence: sirtuins in aging and neurodegeneration. *Neuron* **58**: 10-14.

Gielen PR, Schulte BM, Kers-Rebel ED, Verrijp K, Petersen-Baltussen HM, ter Laan M, Wesseling P, Adema GJ. 2015. Increase in both CD14-positive and CD15-positive myeloid-derived suppressor cell subpopulations in the blood of patients with glioma but predominance of CD15-positive myeloid-derived suppressor cells in glioma tissue. *J Neuropathol Exp Neurol* **74**: 390-400.

Gieryng A, Pszczolkowska D, Bocian K, Dabrowski M, Rajan WD, Kloss M, Mieczkowski J, Kaminska B. 2017a. Immune microenvironment of experimental rat C6 gliomas resembles human glioblastomas. *Sci Rep* **7**: 17556.

Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. 2017b. Immune microenvironment of gliomas. *Lab Invest* **97**: 498-518.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER et al. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**: 841-845.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T. 2013. Origin and differentiation of microglia. *Front Cell Neurosci* **7**: 45.

Gorisch SM, Wachsmuth M, Toth KF, Lichter P, Rippe K. 2005. Histone acetylation increases chromatin accessibility. *J Cell Sci* **118**: 5825-5834.

Grauwet K, Chiocca EA. 2016. Glioma and microglia, a double entendre. *Nature Immunology* **17**: 1240.

Gwak HS, Park HJ. 2017. Developing chemotherapy for diffuse pontine intrinsic gliomas (DIPG). *Crit Rev Oncol Hematol* **120**: 111-119.

Hajji N, Wallenborg K, Vlachos P, Füllgrabe J, Hermanson O, Joseph B. 2010. Opposing effects of hMOF and SIRT1 on H4K16 acetylation and the sensitivity to the topoisomerase II inhibitor etoposide. *Oncogene* **29**: 2192-2204.

Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. 2002. Identification of a factor that links apoptotic cells to phagocytes. *Nature* **417**: 182-187.

Hanisch UK. 2002. Microglia as a source and target of cytokines. *Glia* **40**: 140-155.

Hashizume R. 2017. Epigenetic Targeted Therapy for Diffuse Intrinsic Pontine Glioma. in *Neurol Med Chir (Tokyo)*, pp. 331-342.

Hashizume R, Andor N, Ihara Y, Lerner R, Gan H, Chen X, Fang D, Huang X, Tom MW, Ngo V et al. 2014. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med* **20**: 1394-1396.

Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L et al. 2005. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* **352**: 997-1003.

Hezroni H, Tzchori I, Davidi A, Mattout A, Biran A, Nissim-Rafinia M, Westphal H, Meshorer E. 2011. H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells. *Nucleus* **2**: 300-309.

Hisahara S, Chiba S, Matsumoto H, Tanno M, Yagi H, Shimohama S, Sato M, Horio Y. 2008. Histone deacetylase SIRT1 modulates neuronal differentiation by its nuclear translocation. *Proc Natl Acad Sci U S A* **105**: 15599-15604.

Horikoshi N, Kumar P, Sharma GG, Chen M, Hunt CR, Westover K, Chowdhury S, Pandita TK. 2013. Genome-wide distribution of histone H4 Lysine 16 acetylation sites and their relationship to gene expression. *Genome Integr* **4**: 3.

Horten BC, Basler GA, Shapiro WR. 1981. Xenograft of human malignant glial tumors into brains of nude mice. A histopatholgical study. *J Neuropathol Exp Neurol* **40**: 493-511.

Huszthy PC, Daphu I, Niclou SP, Stieber D, Nigro JM, Sakariassen PO, Miletic H, Thorsen F, Bjerkvig R. 2012. In vivo models of primary brain tumors: pitfalls and perspectives. *Neuro Oncol* **14**: 979-993.

Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG, Batchelor TT. 2007. Angiogenesis in brain tumours. *Nat Rev Neurosci* **8**: 610-622.

Jimenez A, Zu W, Rawe VY, Pelto-Huikko M, Flickinger CJ, Sutovsky P, Gustafsson JA, Oko R, Miranda-Vizuete A. 2004. Spermatocyte/spermatid-specific thioredoxin-3, a novel Golgi apparatus-associated thioredoxin, is a specific marker of aberrant spermatogenesis. *J Biol Chem* **279**: 34971-34982.

Jones C, Karajannis MA, Jones DTW, Kieran MW, Monje M, Baker SJ, Becher OJ, Cho YJ, Gupta N, Hawkins C et al. 2017. Pediatric high-grade glioma: biologically and clinically in need of new thinking. in *Neuro Oncol*, pp. 153-161.

Kavanagh E, Rodhe J, Burguillos MA, Venero JL, Joseph B. 2014. Regulation of caspase-3 processing by cIAP2 controls the switch between pro-inflammatory activation and cell death in microglia. in *Cell Death Dis*, pp. e1565-.

Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Holscher C et al. 2013. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* **16**: 273-280.

Kierdorf K, Prinz M. Microglia in steady state. in *J Clin Invest*, pp. 3201-3209.

Koch CM, Andrews RM, Flicek P, Dillon SC, Karaoz U, Clelland GK, Wilcox S, Beare DM, Fowler JC, Couttet P et al. 2007. The landscape of histone modifications across 1% of the human genome in five human cell lines. *Genome Res* **17**: 691-707.

Kooistra SM, Helin K. 2012. Molecular mechanisms and potential functions of histone demethylases. *Nat Rev Mol Cell Biol* **13**: 297-311.

Langley RR, Fidler IJ. 2011. The seed and soil hypothesis revisited - the role of tumor-stroma interactions in metastasis to different organs. *Int J Cancer* **128**: 2527-2535.

Lapointe S, Perry A, Butowski NA. 2018. Primary brain tumours in adults. *Lancet* **392**: 432-446.

Lee DL, Sasser JM, Hobbs JL, Boriskie A, Pollock DM, Carmines PK, Pollock JS. 2005. Posttranslational regulation of NO synthase activity in the renal medulla of diabetic rats. *Am J Physiol Renal Physiol* **288**: F82-90.

Li W, Graeber MB. 2012. The molecular profile of microglia under the influence of glioma. *Neuro Oncol* **14**: 958-978.

Li X, Corsa CA, Pan PW, Wu L, Ferguson D, Yu X, Min J, Dou Y. 2010. MOF and H4 K16 acetylation play important roles in DNA damage repair by modulating recruitment of DNA damage repair protein Mdc1. *Mol Cell Biol* **30**: 5335-5347.

Lillig CH, Holmgren A. 2007. Thioredoxin and related molecules--from biology to health and disease. *Antioxid Redox Signal* **9**: 25-47.

Lin GL, Nagaraja S, Filbin MG, Suva ML, Vogel H, Monje M. 2018. Non-inflammatory tumor microenvironment of diffuse intrinsic pontine glioma. *Acta Neuropathol Commun* **6**: 51.

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. 2007. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* **114**: 97-109.

Lu L, Li L, Lv X, Wu XS, Liu DP, Liang CC. 2011. Modulations of hMOF autoacetylation by SIRT1 regulate hMOF recruitment and activities on the chromatin. *Cell Res* **21**: 1182-1195.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**: 251-260.

Mack SC, Hubert CG, Miller TE, Taylor MD, Rich JN. 2015. An epigenetic gateway to brain tumor cell identity. *Nature Neuroscience* **19**: 10-19.

Mammana S, Fagone P, Cavalli E, Basile MS, Petralia MC, Nicoletti F, Bramanti P, Mazzon E. 2018. The Role of Macrophages in Neuroinflammatory and Neurodegenerative Pathways of Alzheimer's Disease, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis: Pathogenetic Cellular Effectors and Potential Therapeutic Targets. *Int J Mol Sci* **19**.

Markovic DS, Glass R, Synowitz M, Rooijen N, Kettenmann H. 2005. Microglia stimulate the invasiveness of glioma cells by increasing the activity of metalloprotease-2. *J Neuropathol Exp Neurol* **64**: 754-762.

Markovic DS, Vinnakota K, Chirasani S, Synowitz M, Raguet H, Stock K, Sliwa M, Lehmann S, Kalin R, van Rooijen N et al. 2009. Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. *Proc Natl Acad Sci U S A* **106**: 12530-12535.

Marvel D, Gabrilovich DI. 2015. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest* **125**: 3356-3364.

McIlwain DR, Berger T, Mak TW. 2013. Caspase Functions in Cell Death and Disease.

Melguizo C, Prados J, González B, Ortiz R, Concha A, Alvarez PJ, Madeddu R, Perazzoli G, Oliver JA, López R et al. 2012. MGMT promoter methylation status and MGMT and CD133 immunohistochemical expression as prognostic markers in glioblastoma patients treated with temozolomide plus radiotherapy. in *J Transl Med*, p. 250.

Mellert HS, McMahon SB. 2009. hMOF, a KAT(8) with many lives. *Mol Cell* **36**: 174-175.

Michan S, Sinclair D. 2007. Sirtuins in mammals: insights into their biological function. *Biochem J* **404**: 1-13.

Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M. 2007. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* **10**: 1544-1553.

Misuraca KL, Hu G, Barton KL, Chung A, Becher OJ. 2016. A Novel Mouse Model of Diffuse Intrinsic Pontine Glioma Initiated in Pax3-Expressing Cells12. in *Neoplasia*, pp. 60-70.

Mohammad F, Weissmann S, Leblanc B, Pandey DP, Højfeldt JW, Comet I, Zheng C, Johansen JV, Rapin N, Porse BT et al. 2017. EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nature Medicine*.

Monje M, Mitra SS, Freret ME, Raveh TB, Kim J, Masek M, Attema JL, Li G, Haddix T, Edwards MS et al. 2011. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proc Natl Acad Sci U S A* **108**: 4453-4458.

Müller A, Brandenburg S, Turkowski K, Müller S, Vajkoczy P. 2015. Resident microglia, and not peripheral macrophages, are the main source of brain tumor mononuclear cells. *Int J Cancer* **137**: 278-288.

Nasrin N, Kaushik VK, Fortier E, Wall D, Pearson KJ, de Cabo R, Bordone L. 2009. JNK1 phosphorylates SIRT1 and promotes its enzymatic activity. *PLoS One* **4**: e8414.

Nayak D, Roth TL, McGavern DB. 2014. Microglia development and function. *Annu Rev Immunol* **32**: 367-402.

Nimmerjahn A, Kirchhoff F, Helmchen F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* **308**: 1314-1318.

Outeiro TF, Marques O, Kazantsev A. 2008. Therapeutic role of sirtuins in neurodegenerative disease. *Biochim Biophys Acta* **1782**: 363-369.

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L et al. 2011. Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**: 1456-1458.

Perng P, Lim M. 2015. Immunosuppressive Mechanisms of Malignant Gliomas: Parallels at Non-CNS Sites. *Front Oncol* **5**: 153.

Piunti A, Hashizume R, Morgan MA, Bartom ET, Horbinski CM, Marshall SA, Rendleman EJ, Ma Q, Takahashi YH, Woodfin AR et al. 2017. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med* **23**: 493-500.

Ransohoff RM. 2016. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci* **19**: 987-991.

Rea S, Xouri G, Akhtar A. 2007. Males absent on the first (MOF): from flies to humans. *Oncogene* **26**: 5385-5394.

Revollo JR, Li X. 2013. The ways and means that fine tune Sirt1 activity. *Trends Biochem Sci* **38**: 160-167.

Ridler C. 2017. Neuro-oncology: New therapeutic targets for diffuse intrinsic pontine glioma. *Nature Reviews Neurology* **13**: 196.

Rougeulle C, Chaumeil J, Sarma K, Allis CD, Reinberg D, Avner P, Heard E. 2004. Differential histone H3 Lys-9 and Lys-27 methylation profiles on the X chromosome. *Mol Cell Biol* **24**: 5475-5484.

Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou CL, Ransohoff RM, Charo IF. 2010. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS One* **5**: e13693.

Salter MW, Stevens B. 2017. Microglia emerge as central players in brain disease. *Nature Medicine* **23**: 1018.

Sarkar S, Doring A, Zemp FJ, Silva C, Lun X, Wang X, Kelly J, Hader W, Hamilton M, Mercier P et al. 2013. Therapeutic activation of macrophages and microglia to suppress brain tumor-initiating cells. *Ann Neurosci* **20**: 154.

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B. 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* **74**: 691-705.

Schneider R, Grosschedl R. 2007. Dynamics and interplay of nuclear architecture, genome organization, and gene expression. *Genes Dev* **21**: 3027-3043.

Schones DE, Cui K, Cuddapah S, Roh TY, Barski A, Wang Z, Wei G, Zhao K. 2008. Dynamic regulation of nucleosome positioning in the human genome. *Cell* **132**: 887-898.

Shapiro WR, Basler GA, Chernik NL, Posner JB. 1979. Human brain tumor transplantation into nude mice. *J Natl Cancer Inst* **62**: 447-453.

Shen X, Venero JL, Joseph B, Burguillos MA. 2018. Caspases orchestrate microglia instrumental functions. *Prog Neurobiol* **171**: 50-71.

Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K. 2014. Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. *J Neurosci* **34**: 2231-2243.

Sielska M, Przanowski P, Wylot B, Gabrusiewicz K, Maleszewska M, Kijewska M, Zawadzka M, Kucharska J, Vinnakota K, Kettenmann H et al. 2013. Distinct roles of CSF family cytokines in macrophage infiltration and activation in glioma progression and injury response. *J Pathol* **230**: 310-321.

Sousa C, Golebiewska A, Poovathingal SK, Kaoma T, Pires-Afonso Y, Martina S, Coowar D, Azuaje F, Skupin A, Balling R et al. 2018. Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures. in *EMBO Rep*.

Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C et al. 2007. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* **318**: 287-290.

Stylli SS, Luwor RB, Ware TM, Tan F, Kaye AH. 2015. Mouse models of glioma. *J Clin Neurosci* **22**: 619-626.

Svotelis A, Bianco S, Madore J, Huppé G, Nordell-Markovits A, Mes-Masson AM, Gévry N. 2011. H3K27 demethylation by JMJD3 at a poised enhancer of anti-apoptotic gene BCL2 determines ERα ligand dependency. in *EMBO J*, pp. 3947-3961.

Szulzewsky F, Pelz A, Feng X, Synowitz M, Markovic D, Langmann T, Holtman IR, Wang X, Eggen BJ, Boddeke HW et al. 2015. Glioma-associated microglia/macrophages display an expression profile different from M1 and M2 polarization and highly express Gpnmb and Spp1. *PLoS One* **10**: e0116644.

Tam WY, Ma CHE. 2014. Bipolar/rod-shaped microglia are proliferating microglia with distinct M1/M2 phenotypes. in *Sci Rep*.

Tang Y, Li T, Li J, Yang J, Liu H, Zhang XJ, Le W. 2014. Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease. *Cell Death Differ* **21**: 369-380.

Tie F, Banerjee R, Stratton CA, Prasad-Sinha J, Stepanik V, Zlobin A, Diaz MO, Scacheri PC, Harte PJ. 2009. CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycomb silencing. *Development* **136**: 3131-3141.

Tremblay ME, Lowery RL, Majewska AK. 2010. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol* **8**: e1000527.

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T. 2013. Layer V cortical neurons require microglial support for survival during postnatal development. *Nat Neurosci* **16**: 543-551.

Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM et al. 2006. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **439**: 871-874.

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. 2009. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci* **29**: 3974-3980.

Wang H, Lathia JD, Wu Q, Wang J, Li Z, Heddleston JM, Eyler CE, Elderbroom J, Gallagher J, Schuschu J et al. 2009. Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. *Stem Cells* **27**: 2393-2404.

Wei J, Gabrusiewicz K, Heimberger A. 2013. The controversial role of microglia in malignant gliomas. *Clin Dev Immunol* **2013**: 285246.

Weller M, Wick W, Aldape K, Brada M, Berger M, Pfister SM, Nishikawa R, Rosenthal M, Wen PY, Stupp R et al. 2015. Glioma. *Nature Reviews Disease Primers* **1**: 15017.

Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, Weller M, Wiendl H. 2003. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. *Cancer Res* **63**: 7462-7467.

Wong ET, Hess KR, Gleason MJ, Jaeckle KA, Kyritsis AP, Prados MD, Levin VA, Yung WK. 1999. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol* **17**: 2572-2578.

Wu A, Wei J, Kong LY, Wang Y, Priebe W, Qiao W, Sawaya R, Heimberger AB. 2010. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol* **12**: 1113-1125.

Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ et al. 2009. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* **360**: 765-773.

Yang H, Zhang W, Pan H, Feldser HG, Lainez E, Miller C, Leung S, Zhong Z, Zhao H, Sweitzer S et al. 2012. SIRT1 activators suppress inflammatory responses through promotion of p65 deacetylation and inhibition of NF-κB activity. *PLoS One* **7**: e46364.

Ye J, Liu Z, Wei J, Lu L, Huang Y, Luo L, Xie H. 2013. Protective effect of SIRT1 on toxicity of microglial-derived factors induced by LPS to PC12 cells via the p53-caspase-3-dependent apoptotic pathway. *Neurosci Lett* **553**: 72-77.

Ying L, Hofseth LJ. 2007. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res* **67**: 1407-1410.

Zhang F, Wang S, Gan L, Vosler PS, Gao Y, Zigmond MJ, Chen J. 2011. Protective effects and mechanisms of sirtuins in the nervous system. *Prog Neurobiol* **95**: 373-395.

Zhang J, Sarkar S, Cua R, Zhou Y, Hader W, Yong VW. 2012. A dialog between glioma and microglia that promotes tumor invasiveness through the CCL2/CCR2/interleukin-6 axis. *Carcinogenesis* **33**: 312-319.

Zhao L, Wang DL, Liu Y, Chen S, Sun FL. 2013. Histone acetyltransferase hMOF promotes S phase entry and tumorigenesis in lung cancer. *Cell Signal* **25**: 1689-1698.